

VOL. 37, PART 3

SEPTEMBER 1954

The British Mycological Society

(Recognosce notum, ignotum inspice)

TRANSACTIONS

Edited by

P. H. GREGORY and G. C. AINSWORTH



CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE, N.W.1

NEW YORK: 32 EAST 57TH STREET, 22

OFFICERS AND COUNCIL FOR 1954

President

S. D. GARRETT, M.A., D.I.C., Sc.D.

Vice-Presidents

W. D. GRADDON, B.Sc., A.M.I.Chem.E.

C. T. INGOLD, D.Sc., F.L.S.

✠ A. A. PEARSON, F.L.S.

Secretary

J. WEBSTER, B.Sc.

Department of Botany, The University, Sheffield, 10

Foray Secretary

F. B. HORA, Ph.D.

Department of Botany, The University, Reading, Berks.

Plant Pathology Secretary

C. C. V. BATTS, B.Sc., Ph.D.

National Institute of Agricultural Botany, Huntingdon Road, Cambridge

Treasurer

W. BUDDIN, M.A.

'Chiltern Court', St Peter's Avenue, Caversham, Reading, Berks.

Editors

P. H. GREGORY, D.Sc., D.I.C., F.L.S.

Department of Botany, Imperial College of Science and Technology, London, S.W. 7

G. C. AINSWORTH, B.Sc., Ph.D., F.L.S.

Hatherly Laboratories, University College, Prince of Wales Road, Exeter

Council

The OFFICERS with the following in addition:

P. W. BRIAN

J. COLHOUN

C. L. DUDDINGTON

M. B. ELLIS

J. T. MANNERS

J. T. PALMER

B. E. PLUNKETT

G. SMITH

Mrs M. P. TOPPING

B. BARNES, Prof. W. BROWN, A. D. COTTON, Prof. Dame HELEN GWYNNE-VAUGHAN, J. RAMSBOTTOM, E. W. SWANTON, Miss E. M. WAKEFIELD,

M. WILSON

PLANT PATHOLOGY COMMITTEE

Chairman: C. J. HICKMAN, Ph.D.

J. E. CROSSE

S. H. CROWDY

H. F. DOVASTON

P. H. GREGORY

J. M. HIRST

J. E. E. JENKINS

R. A. LELLIOT

E. LESTER

N. F. ROBERTSON

Miss G. M. WATERHOUSE

THE PRESIDENT, the SECRETARY and the PLANT PATHOLOGY SECRETARY *ex officio*

FORAY COMMITTEE

Mrs N. MONTGOMERY, D. A. REID, G. SMITH

The FORAY SECRETARY and the SECRETARY *ex officio*

Recorder: (new British species and records)

M. B. ELLIS, B.Sc., Ph.D.

Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey

The subscription price is 50s. per volume (postfree), or \$8.50 in U.S.A., payable in advance. Subscriptions may be sent to any Bookseller, or to the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1.

Enquiries from the U.S.A. should be addressed to the Cambridge University Press, American Branch, 32 East 57th Street, New York 22.

GLIOCLADIUM ROSEUM BAIN., AND ITS SYNONYMS

By IVOR ISAAC

University College of Swansea

(With 10 Text-figures)

Nine different isolates, including *Verticillium foëxii*, *V. pulverulentum*, variants of both of these, *V. rhizophagum*, three cultures of *Gliocladium roseum*, and one unidentified fungus from soil, all produce both verticillate and penicillate conidiophores.

The conidia, enveloped in mucilage, are abstricted from the apices of the phialides, and in the verticillate conidiophores are aggregated by the mucilage into spherical globules, while in the penicillate ones, joined end to end they form long chains.

The mycelium consists of septate branching hyphae which when developed subaerially become organized into 'ropes', but when prostrate remain simple. The mycelium and spore masses are white when the cultures are grown in the dark, but when exposed to daylight they become salmon-pink. Hyaline sclerotium- and chlamydospore-like bodies are formed in the prostrate mycelium.

The optimum temperature for growth of all the isolates in culture was 25.0° C., although quite good growth occurred throughout the range from 20.0 to 35.0° C. The optimum pH value ranged from 6.4 to 8.0. Growth was good on sucrose, dextrose, maltose and glycerine media, but was most rapid on maltose. Variation of the nitrogen source indicated that all the isolates had a marked preference for media containing sodium nitrate.

Not one of the fungi was pathogenic to any of a wide range of plants normally susceptible to *Verticillium*, and none of them parasitized the potato tubers and tomato fruits tested.

It is concluded that these nine isolates should all be classified in the one species *Gliocladium roseum* Bain.

INTRODUCTION

When isolating pathogenic species of *Verticillium* from soil both at Cambridge and at Swansea, I frequently observed a fungus characterized by the formation of verticillately branched conidiophores bearing terminal balls of conidia as in the genus *Verticillium*, in addition to the production of penicillately branched conidiophores, at the apices of which spores were held in closely aggregated chains. This fungus also occurred as a saprophyte on plants killed by *V. albo-atrum* and *V. dahliae*. In addition, I received from various mycologists further similar isolates reported to be saprophytic on dead plant tissues. Preliminary examination of the organism suggested that it might be either *V. rhizophagum* or *Gliocladium roseum*. However, the Centraalbureau voor Schimmelcultures, Baarn, tentatively suggested that these two so-called species were probably synonyms, not only with one another, but also with *Verticillium pulverulentum* Gouw. and *V. foëxii* van Beyma. The problem of the relationships of these apparent species is the subject of the present investigation.

The genus *Gliocladium* was established by Corda (1840) with the single species *G. penicilloides*, which produced erect, septate conidiophores,

branching penicillately above, with septate primary and secondary branches and crowned by unicellular conidia borne on the tips of branchlets and held firmly together in a dense mass by a mucilaginous exudate. This new genus obviously closely resembled *Penicillium*, except that in the latter the conidia are not enveloped in mucilaginous material. This resemblance has led to a certain amount of confusion, the same form being placed in one genus or the other according to the vagaries of the investigator.



Fig. 1. Conidiophores and a 'rope' of hyphae of *Verticillium pulverulentum*, drawn by the present writer. ($\times 300$.)

Corda also established another genus, *Clonostachys*, characterized by penicillate conidiophores bearing chains of elliptical conidia, the long axes of which were oblique so that the conidia adhered side to side instead of either end on end as in *Penicillium*, or end on end within a mucilage envelope as in *Gliocladium*.

Bainier (1907) isolated from rotting paper a fungus, which he named *G. roseum*, and which produced two systems of conidiophores, branched verticillately and penicillately, respectively, bearing on their ultimate sterigmata or phialides conidia coated with a gelatinous material and organized into spherical balls on the verticillate branches and into long chains on the penicillate ones.

Thom (1910) reported that he had obtained from Kral, in Prague, a culture of a fungus, *Penicillium roseum* Link, which produced conidiophores branched either irregularly or verticillately and bearing conidia which were aggregated into gelatinous slime balls or masses. Thom (1930) described the conidiophores of *Gliocladium* as penicillate and came to the

conclusion (as did Raper & Thom, 1949) that the fungus obtained from Kral was so similar to that described by Bainier as to justify naming it *G. roseum* (Link?) Bain., synonym *Penicillium roseum* Link, if the development of a mucilaginous envelope to the conidia could be regarded as sufficient basis for separating *Gliocladium* from *Penicillium*, though they had some reservations as to the complete identity of Link's and Bainier's species.



Fig. 2. Photographic reproduction of conidiophores of *Gliocladium roseum*, drawn by Bainier.

Petch (1939) stated that the conidia of *Gliocladium* are not produced in chains, but occur singly in succession at the apex of the phialide, though, instead of becoming detached, they are retained in chain form by the exuding mucilage which surrounds them, while the chains themselves are united into masses by the same mucilaginous exudate. He claimed that in some species, e.g. *G. album*, an occasional phialide at the margin of such a mass diverges so much from the others that its conidia do not unite with the general group, but form a separate spherical mass as in *Cephalosporium*. *Gliocladium*, he asserted, should not be classed with *Penicillium* but with *Cephalosporium* and *Acrostalagmus*, and might be considered as 'an *Acrostalagmus* in which the phialides are all situated at approximately the same level, so that their spore masses come into contact and fuse'.

Foëx (1923) described what he considered to be a new species of *Verticillium*, *V. duboys*, which caused a rot of the potato 'Saucisse' in France. His fungus formed sclerotia which remained light-coloured in contrast to the blackened structures of *V. albo-atrum*, and he described the conidiophores of this form as branched both verticillately and penicillately, a conclusion with which van Beyma Thoe Kingma (1928) agreed. Although Foëx called his fungus a *Verticillium* he debated whether it should not be included in the genus *Acrostalagmus*, while van Beyma, without justification, renamed it *Verticillium foëxii*, despite the fact that it had already appeared in the literature for five years as *V. duboys*.

Gouwentak (1924) isolated from dark patches on tomato fruits, together with species of *Phoma* and of *Fusarium*, a species of *Verticillium*, which she named *V. pulverulentum*, but which, as a result of experiment, she decided was not the original cause of the disease of the fruit. She described her fungus as forming very poorly developed aerial mycelium and as producing whorled conidiophores, the ultimate branches of which bore either spherical balls of conidia or larger masses formed by the fusion of the balls. In the absence of diagrams it is difficult to determine from her descriptions whether she saw the verticillate and penicillate conidiophores, but since she stated that it resembled *V. duboys* Foëx, *Acrostalagmus niveus* and *A. albus*, though differing from them in spore size and colour, the probability is that she did observe the two types of conidiophores.

Tehon & Jacobs (1936), describing a root disease of American elm causing widespread damage to trees in Ohio Valley, Cincinnati and Dayton, found that the causal pathogen produced 'simple *Verticillium*-type of conidiophores' and also conidiophores which 'were complex in the extreme, with definite verticils often repeated, and were terminated by water-bound globes of spores as is characteristic of *Acrostalagmus*'. Since these writers considered that *Acrostalagmus* is not essentially different from *Verticillium* they referred the pathogen to this latter genus and named it *V. rhizophagum*.

It would appear from the descriptions that all these species have in common two types of conidia-bearing structures, viz. verticillate and penicillate conidiophores. An attempt was therefore made to determine whether sufficient distinct and constant differences occur in the morphology, physiology and phytopathogenicity of these respective fungi to justify the retention of the original name for each species. A list of the nine isolates used in the experiments to be described is given in Table 1.

Table 1. *Isolates used*

Name of organism	Source of culture
<i>Gliocladium roseum</i> (1)	Centraalbureau voor Schimmelcultures
<i>G. roseum</i> (2)	Commonwealth Mycological Institute
<i>G. roseum</i> (3)	Commonwealth Mycological Institute
<i>Verticillium foëxii</i>	Centraalbureau voor Schimmelcultures
<i>V. foëxii</i> var.	Centraalbureau voor Schimmelcultures
<i>V. pulverulentum</i>	Centraalbureau voor Schimmelcultures
<i>V. pulverulentum</i> var.	Centraalbureau voor Schimmelcultures
<i>V. rhizophagum</i>	Centraalbureau voor Schimmelcultures
Soil isolate	Experimental plot, University College of Swansea

Var. = a variant forming abundant subaerial mycelium in culture.

MORPHOLOGY OF ISOLATES

All the fungi listed in Table 1 were grown both at room temperature with exposure to normal daylight, and in the darkness of an incubator maintained at 25.0° C. Cultures from both single spores and small portions of



Fig. 3. Photographic reproduction of conidiophores of *Verticillium dubois*, drawn by Foëx.

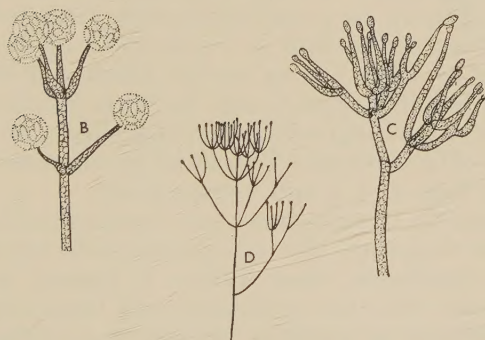


Fig. 4. Photographic reproduction of conidiophores of *Verticillium rhizophagum*, drawn by Tehon & Jacobs.

mycelium were grown on (a) Dox's, Czapek's, potato-extract, potato-dextrose, Brown's, oatmeal-extract, sugar beet-extract, rice-extract, 2 % prune-extract, and 2.5 and 5 % malt-extract agar media in 9 cm. Petri dishes; (b) sterilized potato plugs in test-tubes; and (c) sterilized pieces of elm twigs approximately $1\frac{1}{2}$ in. long and $\frac{1}{2}$ in. diameter in test-tubes. Hanging-drop cultures on Dox's agar were also kept under observation.

In daylight

All the agar cultures grew well and all appeared practically identical, with the exception of *V. foëxii*, *V. pulverulentum* and *Gliocladium roseum* (3), which tended to form sectors developing luxuriant woolly subaerial mycelium on beet-extract, oatmeal-extract, Dox's and 5 % malt-extract media; and also the variants of the first two, which developed abundant white subaerial mycelium with a foam-like appearance. On all the agar media the isolates developed quickly to form (in all except the variants) rather flat and regular colonies with little subaerial mycelium; though where this did occur it appeared as fine 'ropes' of hyphae which, like the prostrate hyphae, were originally white but quickly became salmon-pink in colour. The undersurfaces of the colonies showed a concentric zonation, particularly on potato-dextrose, malt-extract, Dox's, Czapek's and prune-extract agars. After 2-3 days' growth small pink 'balls' which proved, to be masses of conidia, appeared on the upper surfaces of the colonies.

The mycelium consisted of branching septate hyphae, which, in certain of the subaerial growths, became twisted about each other to form rope-like strands (25-30 μ wide) as seen in fig. 1a, and which contained fewer of the oil globules so abundant in the prostrate hyphae.

All the colonies on all the media, except prune-extract agar, spored very profusely, the conidiophores usually developing from the 'ropes' of hyphae, and branching in both a verticillate and a penicillate manner (Fig. 1b and c, respectively), while occasional unbranched conidiophores also occurred (Fig. 1d). The ultimate branches, i.e. the sterigmata or phialides, were usually flask-shaped, and from their apices single, unicellular, hyaline, oval or somewhat spherical conidia were abstricted in succession. At the ends of the verticillate conidiophores these conidia, held together in slime, became aggregated into spherical balls, while the close approximation of the phialides on the penicillate branches resulted in the cohesion of these spore masses, ensheathed in a gelatinous matrix. Very rarely a phialide grew out to form a long hypha which did not produce any conidia (Fig. 1e). Bainier's, Foëx's, and Tehon & Jacobs's illustrations of the conidiophores of the fungi which they described and my drawings of the conidiophores of Gouwentak's *Verticillium pulverulentum* are reproduced for comparison in Figs. 2, 3, 4 and 1 respectively.

The dimensions of the conidiophores and conidia detailed by the authors quoted show some variation, but it is questionable whether comparison between them is justifiable, since Tehon & Jacobs, in their study of *V. rhizophagum*, found that these structures vary markedly within the one species with variations of medium, temperature, light and humidity. I therefore measured conidia and conidiophores from 10-day colonies of all isolates grown under precisely the same conditions on Dox's agar medium, exposed to daylight and kept at normal laboratory temperature (approximately 18-20° C.). No consistent differences could be detected in the dimensions of these structures between any of the nine isolates since in all the conidia measured 3.1-4.8 $\times$ 3.2-8.4 μ ; the conidiophores of all varied from 60 to 200 μ in length and the phialides from 15 to 30 μ .

Foëx (1923) described certain structures in *V. dubois* which he called

sclerotia (Fig. 5). However, he pointed out that they differed from sclerotia of other species of *Verticillium* in that they never became blackened. Tehon & Jacobs (1936) found many hyaline spherical chlamydospores in newly isolated cultures of *V. rhizophagum*, though rarely in old ones. No description

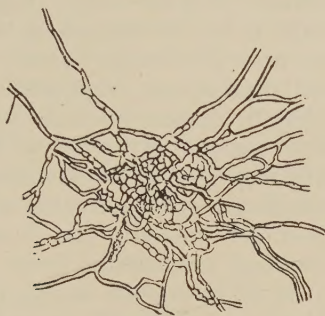


Fig. 5. Photographic reproduction of a sclerotium-like body formed by *Verticillium dubois*, drawn by Foëx.

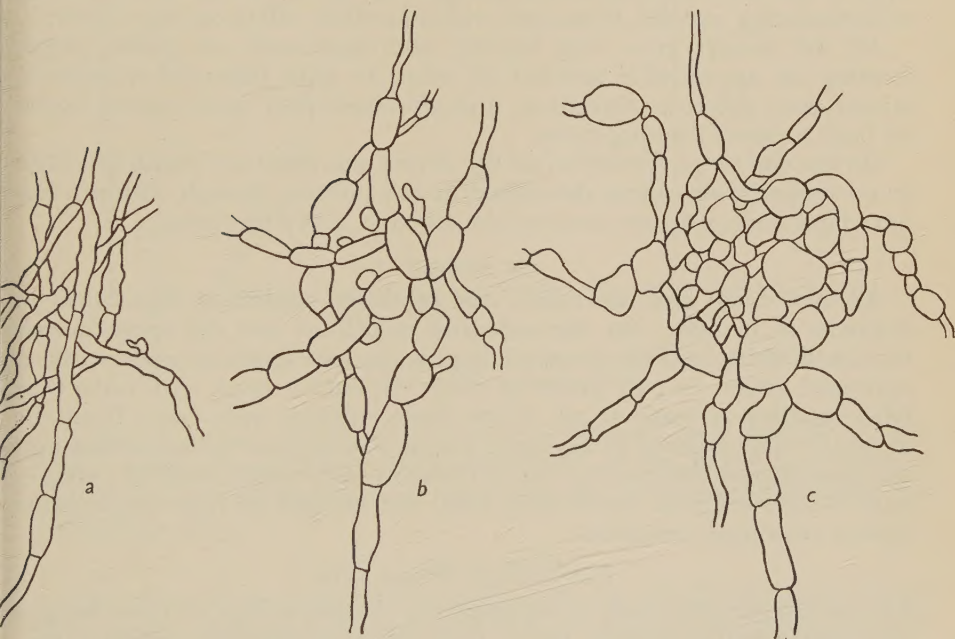


Fig. 6. The development of a 'mycelial knot' of *Gliocladium roseum* from a pure culture on a hanging drop of Dox's agar. *a*, after 4-5, *b*, after 7, and *c*, after 9-10 days' growth respectively. ($\times 450$.)

of similar resting structures occurs in the accounts of the other species under discussion, yet when these were grown on potato-dextrose, beet-extract, malt-extract and oatmeal agars, every one of the nine isolates formed hyaline sclerotium- and chlamydospore-like bodies in the prostrate mycelium. In hanging-drop cultures grown from single spores conidia germinated after 18-24 hr. to form hyphae of uniform diameter in which

oil globules accumulated and in which septa appeared within the first 48 hr. After 4–5 days some hyphae became irregular in places (Fig. 6*a*); occasional ‘cells’ were budded off in terminal and lateral positions. In these swellings oil drops accumulated before they became cut off by septa to resemble chlamydospores ($6\text{--}9 \times 6\text{--}18\mu$) (Fig. 6*b*), but they never developed thick black walls as do corresponding structures in *V. nigrescens* (Isaac, 1949), *V. nubilum* and *V. tricornis* (Isaac, 1953). Where hyphae bearing these hyaline chlamydospores happened to be in close contact the resulting structures (Fig. 6*c*), approximately 30–100 μ diameter appear like those so-called sclerotia depicted by Foëx (Fig. 5). However, these apparent sclerotia merely consisted of intertwining hyphae which in no way became so closely associated with each other as do hyphae forming the true sclerotia of *V. dahliae* and *V. tricornis*, and these may best be described as ‘mycelial knots’ somewhat resembling those reported by Keyworth (1942) and Isaac (1949) in *V. albo-atrum* except that in the latter fungus the hyphal walls were blackened. The difference between these ‘mycelial knots’ and true sclerotia was emphasized by the readiness with which the hyphal strands separated under pressure of the cover-slip or by teasing with dissecting needles, treatment which has little effect on true sclerotia.

All the isolates grew very rapidly and luxuriantly on potato plugs, forming an appreciable amount of white to pink subaerial mycelium, salmon-pink prostrate mycelium, and abundant pink spore masses borne on both types of conidiophores.

On the elm twigs, growth of all the strains was also very rapid, but little or no subaerial mycelium developed in any culture, though within a few days pink balls of spores covered the wood in every test-tube.

In darkness

All these fungi grew as rapidly and produced conidia as abundantly in darkness as in light, but the subaerial mycelium and the spore masses remained white, and the prostrate hyphae, as seen from the undersurface, appeared cream to pale green in colour in each colony with only very faint zonation or none at all. When these cultures were taken from the incubator and exposed to daylight, within 2–3 days all the mycelium and the spore masses assumed the normal salmon-pink colour, and the younger parts of the colonies, developing after the transfer to light showed the typical concentric zonation.

PHYSIOLOGY OF ISOLATES

The technique employed in investigating the physiology of these isolates was similar to that used by Isaac (1949, 1953) in studying certain pathogenic species of *Verticillium*. In each series of experiments four replicate plates were kept and the average measurement of two diameters, at right angles to each other, of all four colonies recorded.

Temperature effects

To compare the relative degree of development of the nine isolates at various temperatures, all were grown on Dox's and on potato-dextrose agar media and incubated at 10.0, 15.0, 20.0, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5 and 40.0° C., respectively.

Only the data for *Gliocladium roseum* (1) and for one of the variants on Dox's are shown in the graph (Fig. 7), since these two are typical of their respective groups, and since similar results were obtained on both media.

The results indicate that, with the exception of *Verticillium foëxii* var. and *V. pulverulentum* var., both of which grew rather slowly, the development of all the colonies at all the various temperatures was very similar. Optimum temperature for all the isolates was 25.0° C., although quite good development occurred from 20.0 to 35.0° C.

Moreover, growth of all isolates at all these temperatures resulted in colonies of the typical form, except in *V. foëxii* var. and *V. pulverulentum* var., which produced the typical fairly abundant white subaerial mycelium at the lower temperatures, but at 30.0° C. and upwards developed only

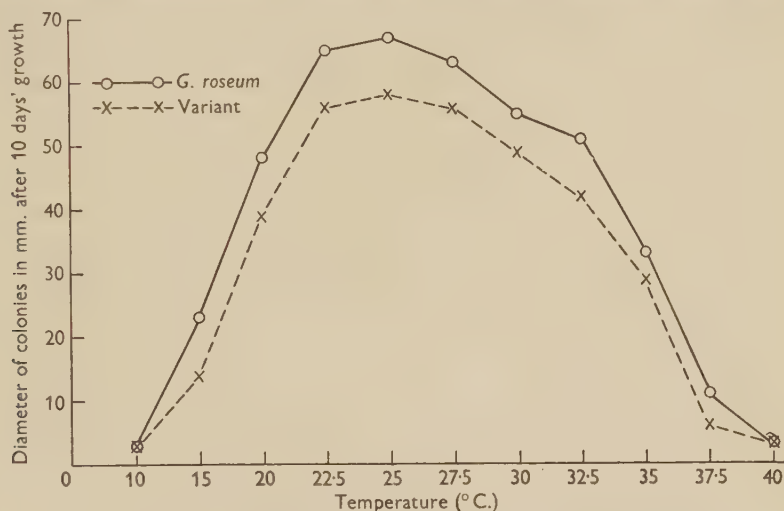


Fig. 7. Influence of temperature upon the growth of *Gliocladium roseum* and a variant on Dox's agar medium.

prostrate hyphae. All the fungi studied produced fewer conidia at these higher temperatures than at the lower range. At the higher range also the verticillate conidiophores were generally far more abundant than the penicillate ones.

pH of media

The effect of the growth of the isolates on the pH of various media was then tested. All these fungi were grown in daylight at laboratory temperature on a number of Petri dish plates of each of the following agar media: potato-extract, potato-dextrose, normal Dox's, Dox's with 0.2 % ammonium nitrate and Dox's with 0.2 % ammonium sulphate, the ammonium salt being substituted in both for the normal sodium nitrate. The initial pH of the two potato media was 6.4 while that of the other three was 5.3. Every day for 10 days the pH of one plate of each medium in which each isolate was growing was tested by the 'drop' method, i.e. using the B.D.H. indicators and comparing the results with the standards in Clark's Table (1923).

The results were very consistent, all the isolates having similar effects upon the media. Normal Dox's and potato-extract media rapidly became very alkaline with a final pH of approximately 8-9; the ammonium nitrate medium became alkaline, though very slowly with a final pH of approximately 6.4; the pH of the potato-dextrose remained constant; the ammonium sulphate medium rapidly became very acid with a final pH of 1.0-1.6 right to the edge of the plate, although here all the fungi appeared as small, irregular, convoluted, fawn-coloured colonies, bearing very few conidiophores.

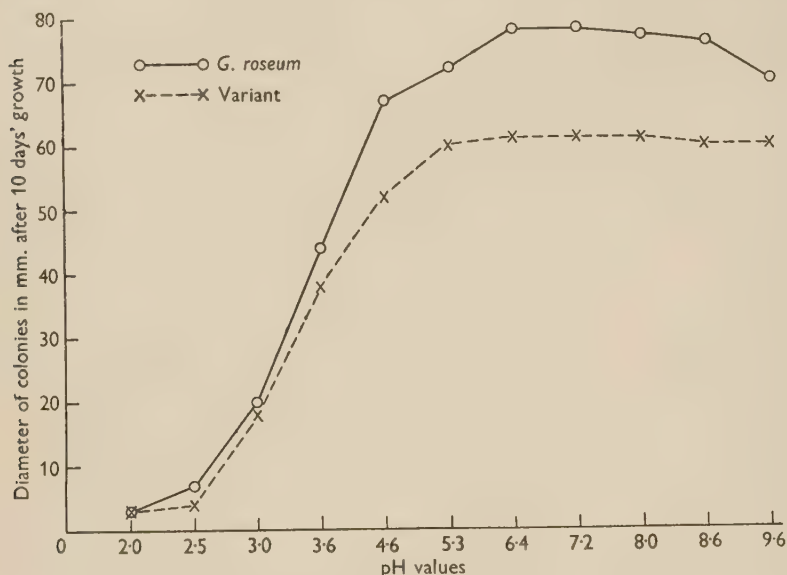


Fig. 8. Influence of initial pH of potato-dextrose agar on the growth of *Gliocladium roseum* and a variant.

Since the effects of all the isolates when grown on various media with a constant initial pH, were so uniform, the results of varying the initial pH were now investigated. All the strains under discussion were grown in series on Dox's and potato-dextrose agars, which had been adjusted to pH's 2.0, 2.5, 3.0, 3.6, 4.6, 5.3, 6.4, 7.2, 8.0, 8.6 and 9.6 by the use of sodium hydroxide and hydrochloric acid, both of normal strength for the Dox's medium, and the same reagents at twice normal for the potato dextrose since this had proved to be naturally buffered. Four plates of each medium at each pH value were prepared for each isolate, and all, after inoculation, were incubated at 25.0° C. for 10 days.

As all the isolates except the variants showed precisely similar responses on the two media, and as the behaviour of the two variants resembled each other closely, only the growth results for *Gliocladium roseum* (1), and for one variant on potato-dextrose agar are shown in Fig. 8.

All the isolates developed well at all initial pH values above 4.6, with a maximum of growth occurring between 6.4 and 8.0. They also resembled each other in showing some development at 2.5 and none at 2.0.

The type of growth was normal for each fungus, except at pH 2.5 on which medium the colonies were small, irregularly shaped, convoluted, pale fawn in colour with very few conidiophores, all of which were verticillate.

Variation in the source of carbon

All the isolates were grown in similar series of four plates on Dox's medium in which the source of carbon was varied by substituting for the normal 1.5 % sucrose, 1, 3 and 5 % of sucrose, dextrose, maltose and glycerine.

The best source of carbon for all the isolates, judged by the rate of growth, proved to be maltose, with dextrose a close second. On all the media except glycerine an increase in the concentration of the carbon compound increased the rate of growth of every isolate. All the fungi on all media grew true to the type normally developed on unmodified Dox's medium.

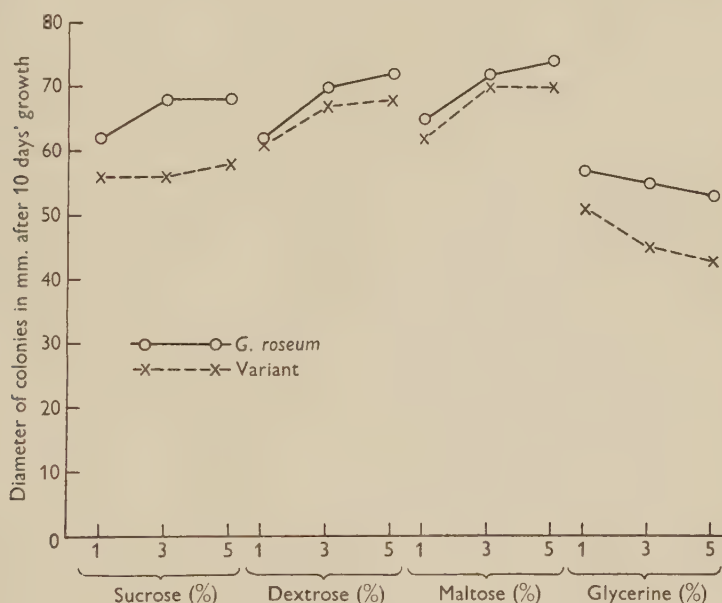


Fig. 9. Influence of the various carbon compounds upon the growth of *Gliocladium roseum* and a variant.

The growth reactions of the main forms were so similar, with slight but consistent differences for the two variants, that only the results for *G. roseum* (1) and one variant are shown in Fig. 9.

Variation in the source of nitrogen

The nine fungi were grown in series of four plates on Dox's medium in which the nitrogen content had been varied by substituting for the normal 0.2 % sodium nitrate 0.1, 0.5 and 1.0 % of asparagine, peptone, sodium nitrate, ammonium nitrate and ammonium sulphate. As the ammonium sulphate medium rapidly became too acid for the growth of these fungi,

three additional similar series were prepared for each isolate in which the 1.0 % ammonium sulphate was buffered with calcium carbonate (0.1, 0.5 and 1.0 % respectively) and, for comparison, a further three series containing a 1.0 % sodium nitrate medium similarly buffered. After inoculation of series of each of these twenty-one media with each isolate all were incubated at 25.0° C.

All the fungi showed a marked preference for the sodium nitrate, and reacted similarly on all the other media, though the two variants grew rather more slowly than did the other seven isolates.

Since the growth of the various isolates was again so very consistent only data for *G. roseum* (1) and one variant are shown in Fig. 10.

The rate of growth and the general morphology of the isolates showed marked similarity on any one medium, but differences occurred on the various media which are worthy of report. On the asparagine media development of all the colonies was normal except for a tendency by *Verticillium foëxii* to saltate frequently. On the concentration of 1.0 % asparagine growth in diameter was not so great, but more subaerial mycelium was generally formed than on both 0.1 and 0.5 %. Heavy sporing occurred on all media, but on 1.0 % all the colonies tended to form more of the *Verticillium*-type of conidiophore than of the *Penicillium*-type.

On 0.1 % peptone all the colonies appeared as flat mats with subaerial mycelium mainly confined to the centres; with an increase in the concentration the proportion of these subaerial hyphae progressively increased, and a corresponding increase in the staling appearance was noticeable, culminating on 1.0 % in undulations, which are so characteristic of marked staling, radiating from the centres of the colonies. Sporing was normal on all media containing peptone.

On the sodium nitrate growth of all the isolates was true to type as on Dox's agar, with the development of slightly more subaerial mycelium as the concentration of this salt increased. Addition of the calcium carbonate had no apparent effect.

On ammonium nitrate media growth occurred as on Dox's, with normal sporing, but on the ammonium sulphate all the colonies appeared as abnormal yeast-like masses. When 0.1 % calcium carbonate was added to the ammonium sulphate some normal growth occurred, but after 3-4 days the margins of the colonies became very convoluted, appeared yeast-like, and growth ceased. With the addition of 0.5 or 1.0 % of calcium carbonate the general morphology of the organisms was typical of that on normal Dox's agar medium, suggesting that these quantities of the carbonate, unlike the 0.1 %, were adequate for the neutralization of the acid formed as a result of the growth of the fungi on an ammonium sulphate medium.

These facts suggest that all the isolates are able to utilize the nitrogen of both the ammonium and the nitrate ions, but that the ammonium sulphate medium, as a result of the absorption of the ammonium ion, eventually became too acid for further growth, unless it was adequately buffered. The fact that the ammonium nitrate media supported steady and normal growth, while their pH remained constant, indicates a probable simultaneous utilization of both ammonium and nitrate ions.

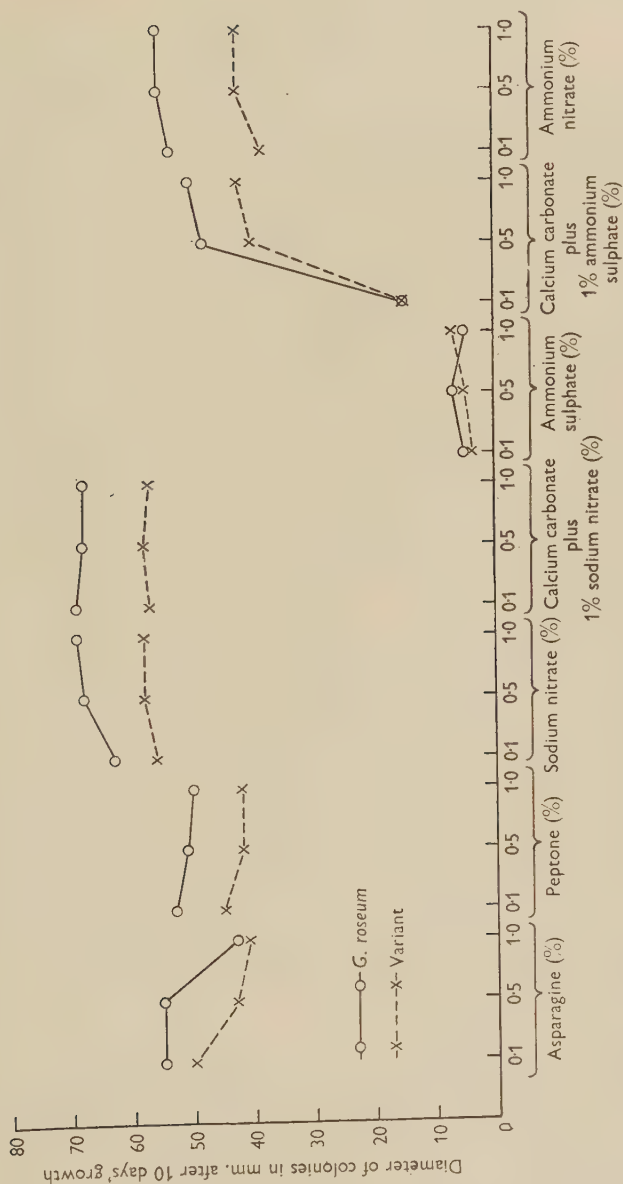


Fig. 10. Influence of various sources of nitrogen upon the growth of *Gliocladium roseum* and a variant.

The evidence so far reported shows clearly that no critical cultural differences exist between *Gliocladium roseum*, *Verticillium foëxii*, *V. pulverulentum* and *V. rhizophagum*, and that the two variants differ from the parent forms only in the production of proportionately more subaerial hyphae and in a consistently slower rate of growth.

STUDIES IN PATHOGENICITY

Foëx (1923) described *V. duboys* as a 'rot-fungus' causing damage to Saucisse potato tubers. Attempts which he made to infect tubers, and thus induce typical *Verticillium* wilt in growing aerial stems, failed, but since he used only four tubers Rudolph (1931) was critical of these results, and suggested that more extensive inoculation experiments were desirable in order to determine whether this organism was similar to *V. albo-atrum* and *V. dahliae* in its effect on potato plants. Tehon & Jacobs (1936) reported that the fungus which they named *V. rhizophagum* was a vascular parasite in American elm. No other report on the pathogenicity of any of these nine isolates was found in the literature, although Gouwentak (1924) stated that *V. pulverulentum* was frequently isolated from infected tomato fruits. Here, however, it was apparently not the cause of the disease, but a secondary invader of tissues rendered moribund by other fungi.

Attempts were therefore made to determine whether these nine isolates were pathogenic to potato (Eclipse), tomato (Kondine Red), salsify, egg-plant, antirrhinum, cucumber, strawberry, sainfoin (common), lupin, carnation and tobacco, which are all normally susceptible to infection by pathogenic species of *Verticillium*. Unfortunately no American elm could be obtained for investigation.

Series of seven plants of each host, grown in John Innes Compost in pots and stood either in cold frames or in the open, were subjected in June to wound inoculations with each of the nine isolates by the insertion of mycelium and spores into wounds made into the xylem of the stems at points from 4 to 6 in. above ground-level. The wounds were bound with 'Elastoplast' to prevent desiccation of the inoculum. Control plants of each host, similarly wounded but not inoculated, were also prepared.

After periods varying from 4 to 5 months none of the plants had wilted, and no trace of mycelium could be found in any living cells of any host, though from the tissues killed by the actual wounding every one of the fungi was subsequently cultured. Therefore it is clear that, though all could exist saprophytically, or persist in the relatively dormant conidial stage, on dead tissues of the different hosts, not one of the isolates is parasitic on any of the plants tested.

Six potato tubers of each of the varieties Majestic, King Edward and Eclipse, and six tomato fruits (Kondine Red) were wound-inoculated with mycelium and spores of each isolate, and all were kept in moist chambers to increase the chances of infection. None of the potato varieties proved to be susceptible, and the living tissues of the tomatoes were also resistant to infection, as Gouwentak had found with *V. pulverulentum*. Nevertheless, dead potato tubers and tomato fruits, killed either by crushing and rotting or by boiling, invariably proved to be most suitable for the saprophytic development of all the isolates.

These tests therefore reveal a consistent lack of pathogenicity on the part of all the isolates, and the results serve to emphasize still further their marked similarity of behaviour.

CONCLUSIONS AND DISCUSSION

On morphological, physiological and phytopathological grounds the nine isolates used are similar and should all be referred to one species.

The question now arises as to what genus and species these forms can appropriately be referred. The development of a gelatinous matrix which envelopes the conidia and binds them into masses prevents the inclusion of these isolates in the genus *Penicillium*, even though some penicillate conidiophores are formed.

The mode of adherence of the conidia, end on end in chains at the apices of the phialides in these nine forms differs from that characteristic of *Clonostachys*. *Acrostalagmus* is frequently discussed in the literature on these species, but it has been conclusively shown by Hoffman (1854) and Carpenter (1918) to be a synonym for *Verticillium*, and therefore does not enter into the present discussion.

There remain for consideration only the genera *Verticillium* and *Gliocladium*. In *Verticillium* the primary and secondary branches of the conidiophores (if present) and the phialides, are invariably whorled, and the members of each whorl are always divergent from each other by a fairly wide angle (Fig. 1*b*). In the organisms under discussion, in addition to verticillate conidiophores, others with primary and secondary branches and phialides closely compacted (Fig. 1*c*), giving a typical penicillate appearance, consistently occurred. As conidiophore form is of primary importance in classifying the Fungi Imperfecti an organism forming penicillate conidiophores should not be included in the genus *Verticillium*. Further, since Corda's *Gliocladium* was established to include fungi forming besides whorled conidiophores others which are not only penicillate but also have their conidia held closely together in a dense mass by a mucilaginous secretion, as do all the isolates under investigation, all these isolates should clearly be referred to this latter genus. Bainier (1907) was the first to ascribe fungi producing these two types of conidiophore to the genus *Gliocladium*, and, since he gave the name *G. roseum* Bain. to his organism, that name should be retained and should include *Penicillium roseum* Link, *Gliocladium roseum* (Link?) Bainier, *Verticillium dubois* Foëx, *V. foëxii* van Beyma, *V. pulverulentum* Gouwentak and *V. rhizophagum* Tehon & Jacobs.

The author is most grateful to Prof. F. A. Mockeridge for advice in the preparation of this paper, to Miss K. Woolfenden for technical assistance, and to the Commonwealth Mycological Institute and the Centraalbureau voor Schimmelcultures, Baarn, for providing certain cultures of fungi.

REFERENCES

- BAINIER, G. (1907). Mycothèque de l'Ecole de Pharmacie. *Bull. Soc. mycol. Fr.* **23**, 111-112.
- CARPENTER, C. W. (1918). Wilt diseases of okra and the *Verticillium* problem. *J. agric. Res.* **12** (9), 529-546.
- CORDA, A. C. I. (1840). *Icones Fungorum*, **4**, 30-31.
- FOËX, E. (1923). Une pourriture verticillienne du tubercle de pomme de terre. *Ann. Épiphyt.* **9** (2), 121-133.
- GOUWENTAK, C. (1924). Eine neue *Verticillium*art. *Meded. phytopath. Lab. Scholten*, **8**, 55-56.
- HOFFMAN, H. (1854). Spermatien bei einem Fadenpilze. *Bot. Ztg.* **12** (15, 16), 249-254, 265-269.
- ISAAC, I. (1949). A comparative study of pathogenic isolates of *Verticillium*. *Trans. Brit. mycol. Soc.* **22**, 139-157.
- ISAAC, I. (1953). A further comparative study of pathogenic isolates of *Verticillium*: *V. nubilum* Pethyb. and *V. tricorpus* sp.nov. *Trans. Brit. mycol. Soc.* **36**, 180-195.
- KEYWORTH, W. G. (1942). *Verticillium* wilt of hop (*Humulus lupulus*). *Ann. appl. Biol.* **29**, 346-357.
- PETCH, T. (1939). *Gliocladium*. *Trans. Brit. mycol. Soc.* **22**, 257-263.
- RAPER, K. B. & THOM, C. (1949). *A manual of the penicillia*. London: Baillière, Tindall and Cox.
- RUDOLPH, B. A. (1931). *Verticillium* Hadromycosis. *Hilgardia*, **5**, 197-362.
- TEHON, L. R. & JACOBS, H. L. (1936). A *Verticillium* root disease of American Elm. *Bull. Res. Dep., The Davey Tree Expert Co., Kent, Ohio*, no. 6.
- THOM, C. (1910). *Cultural studies of species of Penicillium*. Washington: Government Printing Office.
- THOM, C. (1930). *The penicillia*. London: Baillière, Tindall and Cox.
- VAN BEYMA THOE KINGMA, F. H. (1928). Ueber ein Kartoffelfäule verursachendes *Verticillium*, *Verticillium foëxii*, nov.spec. *Meded. phytopath. Lab. Scholten*, **12**, 31-35.

(Accepted for publication 6 June 1953)

CERCOSPORA ELAEIDIS LEAF SPOT OF THE OIL PALM

By W. G. KOVACHICH

*Research Department, Huilever S.A., Léopoldville,
Belgian Congo*

(With Plate 6 and 2 Text-figures)

Cercospora Leaf Spot of the oil palm is described, and a report is given of an inoculation experiment which proved that the disease is caused by *Cercospora elaeidis* Steyaert. Observations on the penetration of the host by the fungus are recorded and it is shown that the lower surface of the leaf is more easily penetrated than the upper surface. Some methods for controlling the disease are suggested.

DESCRIPTION OF THE DISEASE

Cercospora elaeidis has previously been reported on the oil palm (*Elaeis guineensis* Jacq.) in the Belgian Congo by Steyaert (1948), and in Togoland and the Gold Coast by Hughes (1952). The disease condition associated with this fungus has not previously been described nor has the proof of pathogenicity of *Cercospora elaeidis* to the oil palm been published. In view of the prevalence of this disease, it was considered that it should be investigated.

Cercospora Leaf Spot is a disease of young palms. Seedlings in the seed beds frequently show symptoms of a mild infection but the disease is most serious in nursery palms and those which have recently been transplanted to the fields. In the nurseries visited, all palms which had been established for 8 months or more were heavily infected. They normally grow away from the infection within 2 years of being planted out. The disease is not fatal but retards the growth of the palms.

The earliest sign of infection is a pin-point, chlorotic spot when the leaf is viewed by transmitted light. This rapidly becomes a dull brown colour and slightly sunken. When the infection is by a single conidium the spot rarely exceeds 0.5 mm. in diameter and does not increase in size. Conidiophores and conidia of *C. elaeidis* may be observed on the surface of the disease spot shortly after the appearance of the brown colour. Approximately 1 month later, numerous spots appear around the primary spot and this cycle of conidia production and re-infection continues. Coalescence of the spots occurs and the surrounding tissue becomes orange coloured. Finally, the heavily infected areas of the leaflets become desiccated. The surface of heavily infected leaves is normally littered with numerous conidia of the causal fungus. Pl. 6, illustrates a fairly heavy infection of the leaflets.

Internally, during the early stages of infection, hyaline, septate, intercellular mycelium can usually be seen amongst the mesophyll cells. These cells shrink and gummosis of their walls follows. The cuticle in the region of the infection spot is normally brown coloured.

An average, 1-year-old, infected palm shows no infection of the two youngest open leaves. Solitary, scattered spots may be seen on the leaflets of the third leaf, whilst the fourth, fifth, sixth and seventh leaves are all obviously infected with numerous, composite spots on their leaflets. Desiccation of the leaflets usually begins in the eighth leaf. Some palms appear to be highly susceptible to the disease. The youngest, open leaf of these palms is normally free from spotting, but numerous, solitary spots are visible on the second leaf. Leaflets of the third leaf are well infected and show composite spotting, whilst the diseased tissue accounts for approximately half of the leaf surface of the fourth leaf. Desiccation normally begins in the fifth leaf of these palms.

The disease progresses more rapidly in the older, nursery palms when the leaves of neighbouring palms touch, and it is most prevalent during periods of high humidity.

DESCRIPTION OF *CERCOSPORA ELAEDIS* STEYAERT

The fungus is most easily isolated by picking conidia off the conidiophores on infected leaves. It has also been isolated by culturing recently diseased tissue. *C. elaeidis* grows very slowly on malt agar, corn meal agar, prune agar and palm leaf extract agar. Conidia are produced freely on all these media.

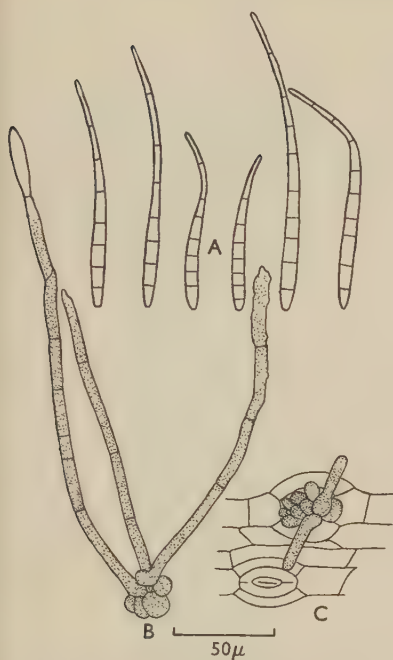
The conidiophores grow out of hyphal knots formed in the stomatal openings of infected leaves and appear in groups of 1-8. They are septate and dark brown coloured, measuring 130-330 μ long and 6-7 μ wide. The conidia are formed at the distal ends of the conidiophores and are hyaline or slightly olivaceous when young and a pale brown colour when older. They are 3- to 9-septate, obclavate with a well-defined basal scar and measure 75-160 μ long and 6-7 μ wide at the widest point. Conidiophores and conidia of *C. elaeidis* are illustrated in Text-fig. 1.

GERMINATION AND PENETRATION

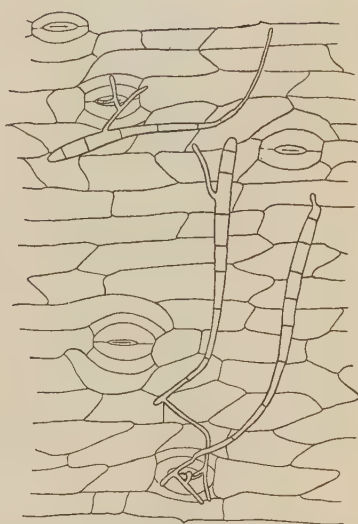
A concentrated spore suspension was obtained by washing conidia off heavily infected leaves. The leaves of a six-leaf seedling were then painted with the spore suspension. Samples of the leaves were taken daily, cleared in pyridine and mounted in lactophenol for examination.

Germination of approximately 8% of the conidia was observed after 24 hr., 50% after 3 days and 75% after 5 days. Normally, one or two germ tubes were produced by each conidium. After 3 days, seven of the sixty-three conidia examined had germ tubes over a stomatal opening, but all seven of these conidia had settled close to, or over, a stoma. As a difference was noticed between the behaviour of the conidia on the upper and lower surfaces of the leaves, separate records were maintained from the fourth day onwards. The results of these observations are tabulated in Table 1. The typical brown spots associated with the disease appeared 21 days after the inoculation of the leaves. In general, the germ tubes of the conidia on the upper surface of the leaf were shorter than those of the lower surface. Text-fig. 2 illustrates the penetration of the stomata by the germ tubes of *C. elaeidis*.

The difference observed between the penetration of the upper and lower surfaces can partly be explained by the difference in the distribution



Text-fig. 1. A, conidia; B, conidiophores; C, hyphal knot and young conidiophores of *C. elaeidis*.



Text-fig. 2. Penetration of the stomata by germ tubes of *C. elaeidis*.

Table 1. Germination and penetration by conidia of *Cercospora elaeidis* painted on the leaves of a six-leaf palm

Days after inoculation	No. of spores not germinated	No. of spores germinated, germ tubes not over stoma	Spores with germ tubes over stoma not penetrated	Spores with germ tubes penetrated stoma
A. Upper surface				
4	12	7	0	0
5	10	9	1	0
6	9	6	3	0
7	26	15	2	0
8	18	22	1	0
9	24	18	5	0
10	34	17	0	1
11	14	15	5	0
12	23	24	4	0
% 7-12 days	51.9	41.4	6.3	0.4
B. Lower surface				
4	20	16	13	0
5	7	12	28	0
6	8	11	18	0
7	15	15	16	0
8	16	11	41	0
9	9	12	26	13
10	15	9	16	11
11	17	18	10	19
12	12	22	26	20
% 7-12 days	22.8	23.5	36.5	17.1

of the stomata on these surfaces. Thus, stomatal counts on the third leaf of six-leaf, 6-month-old and 1-year-old palms showed an average of 13, 8 and 1.8 stomata per mm.², respectively, for the upper surface compared with 66, 79 and 103 for the lower surface. Differences in the environmental conditions are believed to be responsible for the difference in both germination and the length of the germ tubes on the two surfaces.

Attempts to inoculate leaves of young palms with conidia from heavily infected leaves failed when the palms were maintained under the shade of a veranda. This suggests that the penetration of the stomata by the germ tubes is dependent on the degree of opening of the stomata.

PROOF OF PATHOGENICITY

The leaves of twelve, potted seedlings were painted with a concentrated spore suspension obtained by adding sterile water to 6-week-old, pure cultures of *C. elaeidis* and agitating the cultures with a sterile needle. The leaves of twelve other seedlings were painted with sterile water as a control. All the seedlings were maintained in the open air. Typical symptoms of the disease appeared on all the inoculated seedlings within 5 weeks of inoculation. All the control seedlings, except one, were free from infection. The one exception had the tip of one leaf infected where it had, apparently, touched one of the inoculated seedlings.

CONTROL MEASURES

Since the old, desiccated leaves are sources of infection, it is recommended that all dead and nearly dead leaves should be removed and burned. Overcrowding of the palms in the nurseries should be avoided by planting them out in the fields as soon as is practicable. Spraying with a fungicide should be undertaken during the 2 months before and after transplanting and also during periods of increased humidity. The fungicide should be applied from ground-level upwards to ensure a good coverage of the under surface of the leaves.

Preliminary trials, using detached leaves maintained in a moist chamber, showed that 82 % of the conidia had germinated and 11 % had penetrated the leaf after 8 days in the control treatment compared with 12.6 % germinated and no penetration when the leaves were previously treated with a 4-4-40 Bordeaux mixture. As some of the conidia had germinated before they were sprayed on the leaves, it is considered that this mixture is suitable for controlling *C. elaeidis*. Leaves of the oil palm are susceptible to burning by copper fungicides, it is, therefore, essential that only a well-prepared Bordeaux mixture should be used. The substitution of hydrated lime for quicklime in the mixture is recommended.

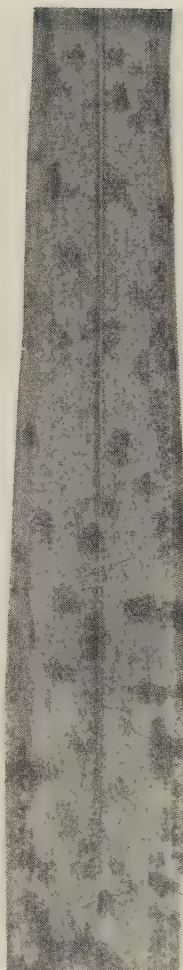
REFERENCES

- HUGHES, S. J. (1952). Fungi of the Gold Coast. I. *Mycol. Paper C.M.I.* no. 48.
 STEYAERT, R. L. (1948). Contribution à l'étude des parasites des végétaux du Congo Belge. *Bull. Soc. Bot. Belg.* **80**, 11-58.

EXPLANATION OF PLATE 6

Leaflets of the oil palm infected by *C. elaeidis*.

(Accepted for publication 18 December 1953)



THE DEVELOPMENT OF SCLEROTIA OF CERTAIN FUNGI

BY BRENDA B. TOWNSEND* AND H. J. WILLETTS†

Department of Botany, University of Bristol

(With 3 Text-figures)

The development of sclerotia of six species of fungi was studied and three different methods of formation were distinguished.

Sclerotial initials of *Rhizoctonia solani* develop by the increased branching and septation of the ordinary hyphae. The mature sclerotia are rather loosely constructed of brown barrel-shaped cells with dense contents.

Sclerotia of *Botrytis allii*, *B. cinerea* and *Sclerotium cepivorum* develop terminally by the repeated dichotomous branching of a hyphal tip followed by increased septation and fusion of the branches.

Mycelial strands of *S. rolfii* and *Sclerotinia gladioli* put out branches which coalesce to form the sclerotia.

INTRODUCTION

Many diseases of economic plants are caused by those fungi which normally produce sclerotia at some time in their life history. These sclerotia are of great biological importance since they may themselves serve as vegetative reproductive bodies, or reproductive structures may develop from them. Moreover, their structure enables them to survive periods of adverse conditions which are too severe for the ordinary vegetative mycelium. Previous investigators have studied the sclerotia of a few plant pathogenic fungi, but, in general, such observations have been subsidiary to the main problem of controlling the disease.

Up to 1853 most sclerotia were considered to represent distinct species. The name *Sclerotium* was introduced by Tode (1790) to designate the single genus in which these supposed species were included. Tulasne (1853), in his work on *Claviceps*, showed that the sclerotium was only a stage in the life history of the fungus.

By 1887 the structure of many mature sclerotia had been studied, though the mode of their development had not been worked out. De Bary (1887) considered that the tissues of the sclerotia, though resembling the ordinary parenchyma of higher plants, were really formed from and consisted of hyphae, and that they owed their parenchymatous structure simply to the firm union of their hyphae, and to the form, expansion and displacement of their cells. De Bary also concluded that mature sclerotia of any species were similar in many important characters. They consist chiefly of a uniform pseudo-parenchymatous centre or medulla, which, with a single exception (*Rhizoctonia solani*), was surrounded by an outer layer of thickened cells forming the rind. De Bary gave no detailed

* Mrs Lee-Whiting.

† Present address: N.A.A.S., Aberystwyth.

descriptions of the development of sclerotia, but simply stated that they arose from a single branch of a mycelial filament, 'which had quickly produced a tuft of many branchlets', or from several adjacent branches of the primary mycelium.

The only detailed study of sclerotial development relates to *Phymotrichum omnivorum*. King & Loomis (1929) showed that the sclerotia were formed by the repeated, irregular division and growth of the cells of large hyphal strands which gave a compact structure of thin-walled, colourless cells, of varying shapes and sizes. Neal, Webster & Gunn (1934) found that these strands consisted of a large central septate hypha, surrounded by two or three layers of elongated thin-walled parallel hyphae, bordered by three or four layers of rather compact thick-walled cells. The enlargement and development of the sclerotium was due to the branching and division of the cells of the central mass, resulting in a mature sclerotium with a distinctly cellular structure. The sclerotia of *Sclerotium rolfsii* (Higgins, 1922), *Rhizoctonia solani* (Palo, 1926), *Typhula gyrans* (Macdonald, 1934), *T. trifolii* (Noble, 1937), *Claviceps purpurea* (Langeron, 1945), *Sclerotium bataticola* (Reichert & Hellinger, 1949), and *Typhula* spp. (Corner, 1950) have all received attention in the last 30 years, but these studies have dealt almost exclusively with the structure of the mature sclerotium. Corner admitted his failure to understand how the sclerotia of *Typhula* sp. arose from the mycelium, and he regarded the development as one of aimless intricate growth.

For the investigation of early stages of sclerotial development direct observation of Petri dish cultures was unsatisfactory, and with teased material the hyphae were easily displaced. The type of slide culture evolved by Vernon (1931) was used for studying the development of the sclerotial initials. The advantages of this method were twofold: it made possible the investigation of the initials without disturbance of the mycelium, and the growth of one particular sclerotial initial could be followed.

Mature sclerotia were fixed in a solution containing four parts of absolute alcohol to one part of glacial acetic acid. After washing with 70 % alcohol, they were dehydrated in 85, 90 % and absolute alcohol and finally with 2:1 and 1:2 mixtures of absolute alcohol and butyl alcohol. The sclerotia were embedded in wax and cut at intervals of 10 μ . The sections were all stained with Delafield's haematoxylin.

Sclerotia of the following fungi were studied: *Rhizoctonia solani* Kühn, *Sclerotium cepivorum* Berk., *Botrytis allii* Munn, *B. cinerea* Fr., *Sclerotinia gladioli* Drayt. and *Sclerotium rolfsii* Sacc. These are described in three categories, according to their modes of development. Earlier work on sclerotial formation can be fitted into such a classification.

1. THE LOOSE TYPE OF SCLEROTIAL DEVELOPMENT

Rhizoctonia solani is the only known example of this type. In the formation of the sclerotial initials there is no definite pattern of organization of the hyphae, and the resulting sclerotium is very loosely constructed.

The ordinary mycelial hyphae of *R. solani* are 7–8 μ wide and are divided by septa at intervals which vary from 54 to 216 μ . The sclerotial initials are formed by the branching of the ordinary mycelium with the

production of much more numerous septa (Fig. 1 *a, b*). These hyphae are considerably thicker than those of the ordinary mycelium, being $20\text{--}22\ \mu$ broad, and their abundant septation results in short, barrel-shaped cells, about $30\text{--}35\ \mu$ long (Fig. 1 *c, d*). These are filled with dense contents and numerous vacuoles. Continued dividing and branching produces a mass



Fig. 1. *Rhizoctonia solani*. (*a*) Early stage in development of sclerotial initial. Adjacent hyphae are beginning to branch and to produce frequent septa. The cells assume a barrel shape. (*b*) The same initial 24 hr. later. (*c*) Cells from the central mass of a mature sclerotium, identity of hyphae largely lost. (*d*) Cells from loosely arranged peripheral part of mature sclerotium showing irregularly septate hyphae. $\times 500$.

of hyphal cells, almost yeast-like in appearance. The sclerotium darkens to reddish brown without any further increase in size.

The mature sclerotium is much less closely interwoven than that of other fungi studied and there are no well-defined zones. A small central part of the sclerotium has a pseudo-parenchymatous appearance (Fig. 1 *c*), but in most of it the hyphal nature can be clearly seen. Towards the margin the hyphae are more loosely arranged, and there is no outer layer of

thickened cells. In the cells of the mature sclerotium there are no vacuoles, but each cell contains six to twelve small darkly staining granules. The dimensions of the cells average $29 \times 18 \mu$ —slightly less than those of the young sclerotial initial, but they are still much broader and shorter than those of the ordinary mycelium. This sclerotial initial increases in size by repeated branching and the formation of new cells. In its mode of development and in the differentiation of the mature structure, the sclerotium of *R. solani* is the simplest of all those studied. Nevertheless, probably by reason of the dense cellular contents it is highly resistant to drought.

2. THE TERMINAL TYPE OF SCLEROTIAL DEVELOPMENT

The sclerotial initials of this group are characterized by a well-defined pattern of branching at the tip of a hypha.

The sclerotial initials of *Botrytis allii* arises by dichotomous branching, followed by the formation of cross-walls (Fig. 2*a-e*). Further branching and septation occur until a mass of hyphae is formed in which the dichotomy is still discernible (Fig. 2*f*). The hyphae then coalesce to produce the appearance of a solid tissue. At first the outlines of the individual cells can still be distinguished, but further details are obscured by darkening of the structure.

The mature sclerotium has three well-defined layers, a 'rind' of six to eight layers of rounded thickened cells of $7-8 \mu$ diameter, a narrow 'cortex', 3-4 cells thick, consisting of thin-walled pseudo-parenchymatous cells with dense contents, and a large central 'medulla' of filamentous hyphae loosely arranged (Fig. 2*g*).

The dimensions of the ordinary vegetative hyphal cells of *B. allii* are $8 \times 54-130 \mu$, but the sclerotial cells produced by dichotomous branching average $8 \times 16-20 \mu$ only. Measurements of the cells in the mature sclerotium thus show that the increase in size of the sclerotium was not due to expansion of the cells formed in the earlier stages.

In all essentials the development of the sclerotial initials and the anatomy of the mature sclerotium of *B. cinerea* are very similar to those of *B. allii*. The 'rind' in *B. cinerea* is narrower and the 'cortex' is broader than the corresponding layers in the sclerotia of *B. allii* (Fig. 2*h*).

The sclerotial initials of *Sclerotium cepivorum* begin development in a similar fashion to those of *Botrytis* spp., but the regularity of the dichotomous branching is lost after the first two divisions, and further divisions produce many branches of approximately equal length (Fig. 2*h-j*). The formation of cross-walls in these hyphae produces chains of short hyphal cells. These branches then intertwine, and a spherical mass of hyphal tissue is produced, consisting of closely packed hyphal cells at the centre and projecting hyphae at the periphery (Fig. 2*k, l*). The central cells coalesce and this area gradually darkens, obscuring further detail (Fig. 2*m*).

In these three fungi, the early stages of sclerotial development resemble the early stages of conidiophore formation, in that dichotomous branching initiates both structures. This suggests that the sclerotia of some fungi may have evolved from conidiophores, the sclerotial initials of *Sclerotium cepivorum* diverging at an earlier stage than those of *Botrytis* spp. All these sclerotia are characterized by coalescence of short hyphal cells at a fairly

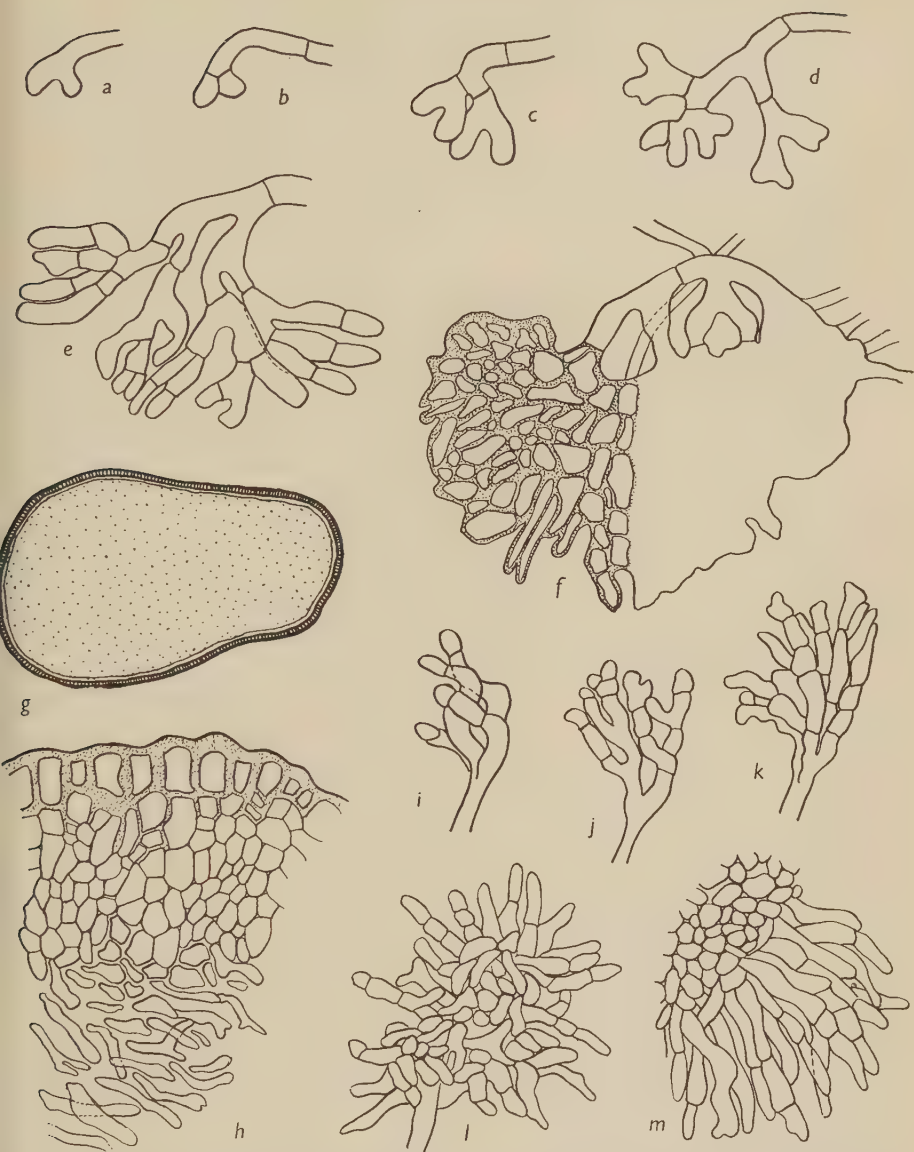


Fig. 2. *Botrytis allii*. (a) Very early stage in development of sclerotial initial showing dichotomy of hyphal tip. (b-d) Further development of same initial, showing successive dichotomous branching and septation. (a-d) Drawn at approximately 4 hr. intervals. (e) Drawn 12 hr. later. (f) Later stage in development of same initial showing coalescence and darkening of hyphae. $\times 500$. (g) Diagram of transverse section of mature sclerotium to show relative thickness of rind, cortex and medulla. (h) *B. cinerea*, part of periphery of mature sclerotium in transverse section. $\times 500$. *Sclerotium cepivorum*. (i-k) Stages in development of sclerotial initial showing that after the initial dichotomy the branching becomes irregular. (l) Later stage in development showing spherical mass surrounded by projecting hyphae. (m) Peripheral hyphae projecting outwards from young sclerotium. $\times 500$.

early stage in their development. The final structure is more complex than the sclerotium of *Rhizoctonia solani* as it shows a clearly differentiated protective rind. This mode of formation, with a compact pseudo-parenchyma, is probably also an advanced character.

3. THE STRAND TYPE OF SCLEROTIAL DEVELOPMENT

The sclerotial initials of this group are formed by the production of numerous side branches along one or more main hyphae or a hyphal strand.

The sclerotial initial of *Sclerotinia gladioli* is the simplest of this type. The formation of the initial commences with the production of numerous small side branches, which arise from one or more main hyphae. If several hyphae are concerned, they lie parallel (Fig. 3*a*), they are slightly thicker than the hyphae of the ordinary vegetative mycelium, are filled with dense contents and are transformed by the production of numerous septa into a chain of short hyphal cells. Short branches are produced by cells of the main hyphae. Some of these lie parallel with the parent hyphae while others grow out at right angles, often branching again before they connect with other hyphae and coalesce. The identity of the hyphae in the central mass becomes lost, and further development is obscured by darkening of the structure (Fig. 3*b, c*). The hyphae at the margin of the central mass continue to branch profusely and coalesce with the others, and this mass of fused hyphae becomes more rounded and less linear in outline. At this stage the sclerotial initial is still connected to the main hypha or hyphae at each end.

The mature sclerotium is small, from 0.1 to 0.3 mm. in diameter, but there are two well-defined zones, a rind (15–20 μ wide) of small thick-walled cells, and a medulla of large thin-walled cells (Fig. 3*d*). The ordinary vegetative hyphae of this fungus are fine, being, like the cells of the rind, about 3 μ wide. However, the medullary cells are 3–4 times this diameter, and the expansion of these cells may account for the distorted appearance of the cells in the rind.

The strand-like nature of the mycelium of *Sclerotium rolfsii* is immediately obvious at the lowest magnification. The strands consist of three to twelve hyphae lying more or less parallel, and show no zonation comparable to that of the strand of *Phymatotrichum omnivorum*. All the hyphae are 3–5 μ wide, thin-walled and sparsely septate in the early stages. The sclerotial initials are formed from these hyphal strands by multiple branching and considerable intertwining of the branches so formed (Fig. 3*e, f*). The initial may arise from a single hyphal strand or from the point of intersection of two strands. When a very young sclerotium of this fungus first assumes its spherical shape, it consists merely of a dense network of hyphae. The amount of septation has markedly increased, but at this stage there is no coalescence of hyphae. The differentiation of the hyphae commences in a slightly older sclerotium (still white, but larger and firmer). There is an outer layer of polyhedral cells, 3–4 μ in diameter, which surrounds a mass of ordinary hyphae, compactly arranged. The 'rind' and 'cortex' become delimited as the sclerotium darkens (Fig. 3*g, h*).

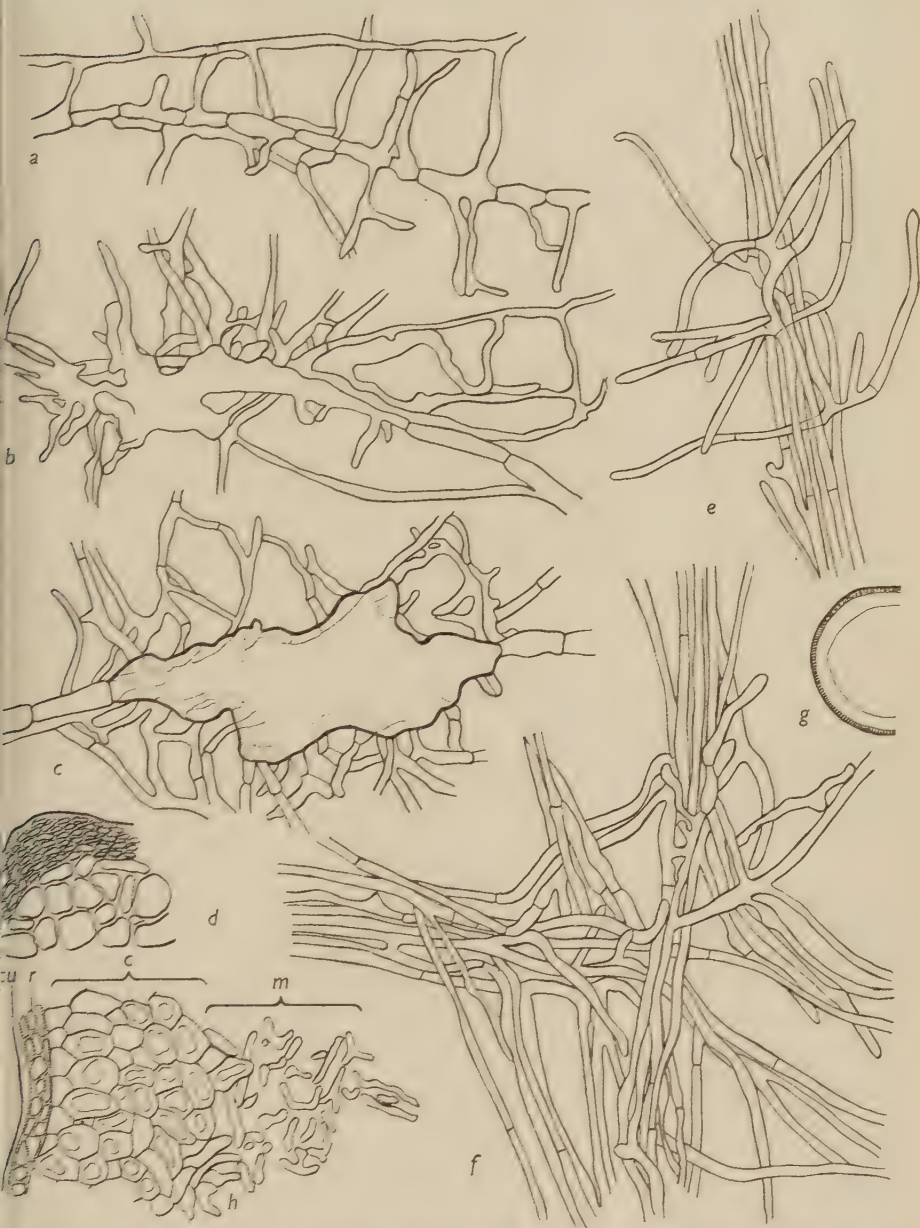


Fig. 3. *Sclerotinia gladioli*. (a-e) Stages in development of sclerotial initial, showing branching of mycelial strand and septation and fusion of branches. (d) Part of periphery of sclerotium. $\times 500$. *Sclerotium rolfsii*. (e, f) Early stages in development of a hyphal strand. $\times 500$. (g) Diagram to show relative thickness of rind, cortex and medulla of mature sclerotium seen in transverse section. (h) Part of transverse section of periphery of mature sclerotium to show cuticle (cu), rind of thick-walled cells (r), cortex (c) of regular pseudo-parenchymatous cells and medulla (m) of irregularly arranged hyphae. $\times 500$.

The mature sclerotium consists of four zones, a fairly thick skin, a 'rind' of thickened cells (2-4 cells broad and flattened tangentially), a 'cortex' of thin-walled cells with densely staining contents (about 6-8 cells broad), and the 'medulla' which consists of ordinary filamentous hyphae, loosely arranged, and filled with dense contents.

The dimensions of the filamentous hyphae of the 'medulla' are similar to those of the ordinary vegetative hyphae, the cells of the 'rind' are approximately $3 \times 7 \mu$, those of the 'cortex' average $14-17 \times 7-11 \mu$ and have considerably enlarged during the growth of the sclerotium. The appearance of the cells of the 'rind' strongly suggests that they have been subject to tangential stress, and the medullary hyphae that were once so compactly arranged are no longer adherent. Thus the growth of this sclerotium has some points of similarity with those of *Typhula gyrans* (Macdonald, 1934) and *T. trifolii* (Noble, 1937), i.e. increase in size of the sclerotia takes place after an outer limiting layer has formed.

The formation of sclerotia of *Sclerotium bataticola* is another example of the 'strand' type of development. Reichert & Hellinger (1949) noted five types of sclerotial formation, but as these were classified by the number of main hyphae on which the initial originated, the division seems rather arbitrary. It has already been stated that one or more main hyphae may participate in the sclerotial development of *Sclerotinia gladioli*, and this variation is probably dependent on the proximity of the sclerotial initials. If neighbouring initials coalesce at an early stage, the resulting sclerotium is simple, if they coalesce at a later stage, a compound sclerotium is produced.

The sclerotium of *Phymatotrichum omnivorum* is probably the most advanced member of this group. It always arises from a mycelial strand which is already differentiated into three zones. The enlargement of the sclerotium is due to the growth of the central mass.

DISCUSSION

The irregular branching of the sclerotial initial of *Rhizoctonia solani* and the failure to coalesce result in a loosely constructed sclerotium which is unique. The mature sclerotia of all other fungi studied are hard, compact structures, protected from desiccation by a more or less well-developed rind. The sclerotium of *R. solani* must owe its considerable resistance to desiccation to the dense contents, pigmentation and relatively impermeable wall of the individual cells.

The 'terminal' type of sclerotial development, typified by that of *Botrytis* spp., suggests that the sclerotia have evolved from spore-bearing structures. It is known that many of them give rise to fruit-bodies under certain conditions. In *Botrytis* spp. the dichotomy continues until the hyphae begin to coalesce, but in *Sclerotium cepivorum* dichotomy is lost at an early stage.

Within the group producing sclerotia by the 'strand' type of development, a series of increasing complexity can be traced. In *Sclerotinia gladioli* and *Sclerotium bataticola* the 'strand' may be only one main hyphae, or it may be two or more that lie parallel. In *S. rolfsii* the sclerotium arises from mycelial strands of five to nine parallel hyphae, and the initial may

originate on a single strand or from the point of intersection of two strands. The strands of *Phymatotrichum omnivorum* consist of a central large hypha, a layer of thin-walled hyphae and an outer layer of thick-walled cells.

There are other differences between the sclerotia produced by these two methods. The growth of the central mass of the terminal type of sclerotium ceases when the limiting layer (the 'rind') has been differentiated, but in the strand type of sclerotium, growth continues and the cells of the limiting layer are stretched tangentially. The 'rinds' of the sclerotia of *Botrytis* spp. are broad and the cortex is narrow. The reverse is true of the sclerotia produced by the strand type of development.

Although the development of the sclerotial initials of *Typhula* has not been worked out, the structure of the mature sclerotia suggests that this genus may be included in the 'strand' group.

The difference in the development of the sclerotia of *Botrytis* spp. and *Sclerotinia gladioli* is noteworthy in view of the heterogeneous collection of fungi which are classified under *Sclerotinia*. It suggests that an examination of vegetative features, such as the development of sclerotia, may possibly provide an aid to the taxonomy of this ill-defined group.

The writers wish to thank Dr L. E. Hawker for suggesting this study, for guidance during its progress and for assistance in the preparation of the manuscript.

REFERENCES

- CORNER, E. J. H. (1950). *A monograph of Clavaria and allied genera*. Oxford.
 DE BARY, A. (1887). *Comparative morphology and biology of the Fungi, Mycetoza and Bacteria*. Oxford.
 HIGGINS, B. B. (1922). Notes on the morphology and systematic relationship of *Sclerotium rolfsii*. *J. Elisha Mitchell sci. Soc.* **37**, 167-172. (Abstr. in *Rev. appl. Mycol.* **1**, 412.)
 KING, C. J. & LOOMIS, H. F. (1929). Experiments on the control of cotton-root rot in Arizona. *J. agric. Res.* **32**, 297-310.
 LANGERON, M. (1945). *Précis de Mycologie*, pp. 45-56. Paris.
 MACDONALD, J. A. (1934). Life history of *Typhula gyrans*. *Ann. appl. Biol.* **21**, 590-596.
 NEAL, D. C., WEBSTER, R. E. & GUNN, K. C. (1934). Morphology and life history of the cotton-root rot fungus in Texas. *J. agric. Res.* **49**, 539-548.
 NOBLE, M. (1937). The morphology and cytology of *Typhula Trifolii*. *Ann. Bot., Lond., N.S.*, **1**, 67-74.
 PALO, M. A. (1926). Rhizoctonia disease of rice. *Phillipp. Agric.* **15**, 361-375. (Abstr. in *Rev. appl. Mycol.* **6**, 252-253.)
 REICHERT, I. & HELLINGER, E. (1949). On the occurrence, morphology and parasitism of *Sclerotium bataticola*. *Palest. J. Bot. Rehovot*, **6**, 107-147. (Abstr. in *Rev. appl. Mycol.* **28**, 84-85.)
 TODE, H. J. (1790-1). *Fungi Mecklenb.* **1** (2).
 TULASNE, L. R. (1853). Mémoire sur l'ergot des Glumacées. *Ann. Sci. nat.*, 3me sér., **20**, 1-56.
 VERNON, T. R. (1931). An improved type of moist chamber for studying fungal growth. *Ann. Bot., Lond.*, **45**, 733-737.

(Accepted for publication 31 July 1953)

MORPHOLOGY AND DEVELOPMENT OF FUNGAL RHIZOMORPHS

By BRENDA B. TOWNSEND*

Department of Botany, University of Bristol

(With 4 Text-figures)

Rhizomorphs of sixteen species of Basidiomycetes were examined and graded into four groups: (1) largely undifferentiated (*Hymenogaster luteus*, *Merulius lacrymans*); (2) showing zones of similar hyphae arranged differently (*Hymenogaster tener*, *Collybia platyphylla*); (3) showing zones of hyphae of different sizes (*Flammula sapinea*, *Crucibulum vulgare*, *Cyathus striatus*, *Psalliota campestris*, *P. arvensis*, *Phallus impudicus*, *Hysterangium nephriticum*); and (4) some of the hyphae with thickened walls (*Lycoperdon pyriforme*, *L. gemmatum*, *Armillaria mellea*, *Marasmius androsaceus*).

Rhizomorphs of *Armillaria mellea* grow best at 25° C. in a humid atmosphere in the dark. The optimum H-ion concentration for their development is pH 5.6. The range over which rhizomorphs develop (pH 3.6–8.0) is slightly narrower than that permitting mycelial growth (pH 3.1–8.8). The ratio of carbon to nitrogen has an effect on rhizomorph formation comparable to that on the development of certain sclerotia; a ratio of three to five parts of glucose to one part of either peptone or asparagine is the most favourable.

MORPHOLOGY OF RHIZOMORPHS

Although the importance of rhizomorphs as agents for the spread of certain root-invading or wood-destroying fungi through the soil has long been recognized, the study of their structure and development has been largely neglected. Rhizomorphs of sixteen species of Basidiomycetes (*Armillaria mellea* (Fr.) Quél., *Marasmius androsaceus* Fr., *Collybia platyphylla* (Pers.) Fr., *Flammula sapinea* Fr., *Psalliota campestris* (L.) Fr., *P. arvensis* (Schaeff.) Fr., *Merulius lacrymans* Fr., *Hysterangium nephriticum* Berk., *Hymenogaster luteus* Vittad., *H. tener* Berk. et Br., *Mutinus caninus* Fr., *Phallus impudicus* Pers., *Lycoperdon pyriforme* Pers., *L. gemmatum* Pers., *Crucibulum vulgare* Tul. and *Cyathus striatus* Pers.) were examined and compared with the few published accounts. It is possible to arrange these in a series according to the degree of differentiation shown.

(1) *Undifferentiated rhizomorphs*

The simplest type of rhizomorph consists of an aggregation of hyphae, more or less homogeneous in structure and arrangement but with a tendency to the development of a firm margin. Garrett (1944) cites *Fomes lignosus* as an example of this simple type.

The simplest rhizomorph studied was that of *Hymenogaster luteus* which consists of narrow, sparsely septate hyphae arranged more or less parallel to the long axis of the rhizomorph. The outer hyphae are rather more

* Mrs Lee-Whiting.

closely interwoven than the central ones but there is no clear differentiation of an outer protective zone.

The rhizomorph of *Merulius lacrymans* consists of irregularly arranged hyphae of uniform diameter (3μ). The peripheral hyphae tend to encircle the strand, and the resulting firm margin is rather more defined than that of *Hymenogaster luteus* but is still not clearly differentiated from the bulk of the hyphae. In view of the well-known ability of the rhizomorphs of this fungus to travel long distances from their food base, the lack of development of specialized conducting and protective hyphae is surprising.

Rhizomorphs of *Mutinus caninus* consist of irregularly arranged, sparsely septate hyphae of uniform diameter ($4-5\mu$). These are very loosely arranged in the centre of the strand, leaving large air spaces, but become gradually more closely interwoven towards the margin which shows no particular orientation of the hyphae. Lateral branches arise from the outer layer, and occasionally two such branches meet and fuse to form a loop.

(2) *Rhizomorphs showing more or less clearly defined zones*

(a) *Zonation due to arrangement of similar hyphae.* The constituent hyphae of the rhizomorphs of *Collybia platyphylla* and *Hymenogaster tener* are of uniform size and structure, but are arranged in four poorly defined zones (Fig. 1a-r). In both fungi the central zone or core is relatively broad. In *Collybia platyphylla* this zone consists of hyphae arranged in a longitudinally elongated network (Fig. 1c, g). This is surrounded by an inner 'cortex' of compactly arranged hyphae running longitudinally with few air spaces (Fig. 1d, h) and an outer cortex of irregularly arranged and loosely interwoven elements (Fig. 1e, i). The whole is surrounded by a few layers of more or less fused hyphae which form a firm sheath (Fig. 1f, j). In *Hymenogaster tener* the central core does not form a network but consists of rather compactly arranged hyphae mostly running in a longitudinal direction (Fig. 1m, p). The cortex consists of irregularly interwoven hyphae, of which the outer zone is more compact (Fig. 1n, o, q, r). The firm outer margin is similar to that of *Collybia platyphylla*, but fusion of the constituent hyphae is more complete so that the individual elements are difficult to distinguish. In both species all the zones contribute to the production of lateral branches.

(b) *Zonation due to differences in size of hyphae.* This type of rhizomorph may show either a central zone in which some or all of the hyphae are broader and more frequently septate than those of the outer layers, as with *Flammula sapinea*, *Crucibulum vulgare*, *Cyathus striatus*, *Psalliota campestris*, and *P. arvensis*, or the central core may consist of compactly arranged narrow hyphae and the outer layer of broader ones, as in *Hysterangium nephriticum* and *Phallus impudicus*.

The simplest example of the first of these groups is *Flammula sapinea*, in which all the hyphae are arranged loosely, more or less parallel to the axis of the rhizomorph, but those of the central core are $5-9\mu$ wide, while those of the outer layer are only $2-3\mu$ wide. Where the two zones grade into one another the large and small types of hyphae are intermixed (Fig. 2a-f). The rhizomorphs of *Crucibulum vulgare* and *Cyathus striatus* have two clearly

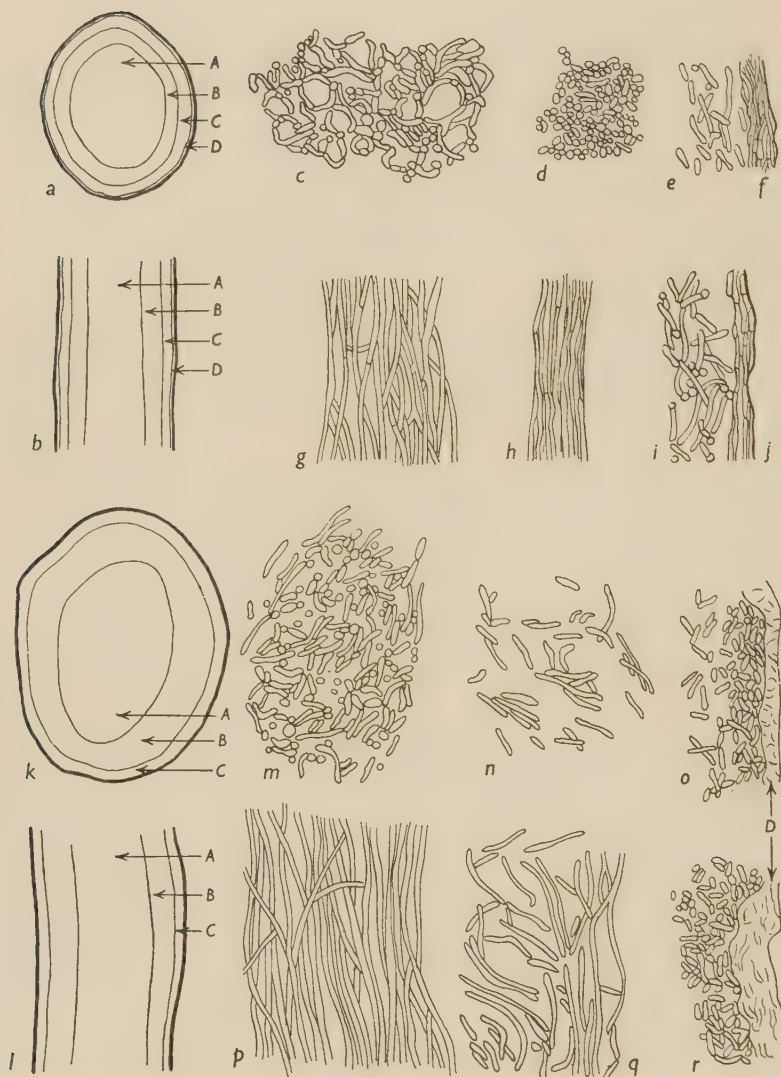


Fig. 1. *Collybia platyphylla*. (a) diagram to show arrangement in transverse section: *A*, central core of narrow hyphae in a reticulate arrangement; *B*, cortical zone of compactly arranged hyphae running longitudinally; *C*, outer cortex of loosely arranged hyphae; *D*, marginal layer of compactly arranged hyphae. (b) to show arrangement in longitudinal section, lettering as in (a). (c-f) hyphae from zones *A-D* respectively as seen in transverse section ($\times 500$). (g-j) hyphae from zones *A-D*, respectively, as seen in longitudinal section ($\times 500$). *Hymenogaster tener*. (k) diagram to show arrangement in transverse section: *A*, central core of narrow hyphae arranged longitudinally; *B*, cortical zone of more loosely interwoven hyphae; *C*, peripheral zone of irregularly arranged hyphae which fuse at extreme edge (*D*) to form firm margin. (l) to show arrangement in longitudinal section, lettering as in *k*. (m-o) hyphae from zones *A-C*, respectively, as seen in transverse section (*D* shown diagrammatically). (p-r) hyphae from zones *A-C*, respectively, as seen in longitudinal section ($\times 500$).

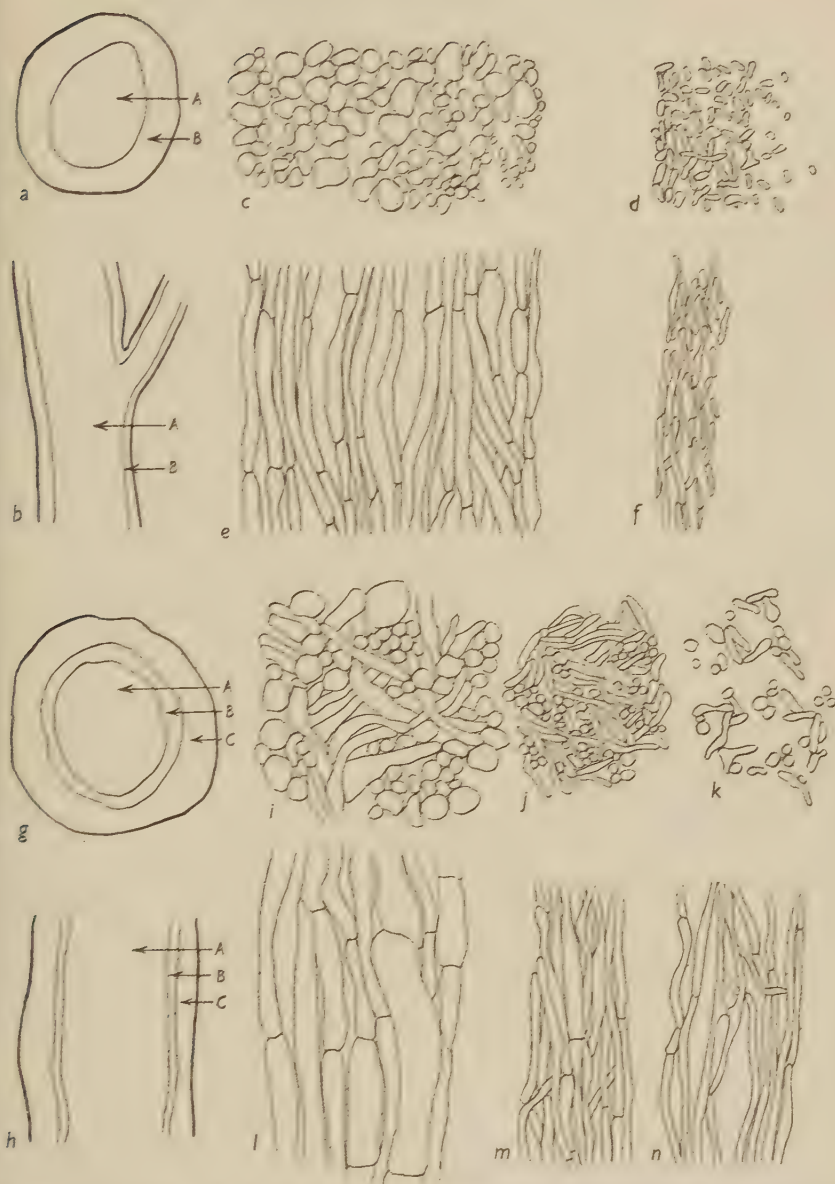


fig. 2. *Flammula capinea*. (a, diagram to show arrangement in transverse section: A, central core of broad hyphae; B, peripheral zone of narrow hyphae. (b, to show arrangement in longitudinal section, lettering as in (a). (c, d, hyphae from zones A, B, respectively, as seen in transverse section ($\times 500$). (e, f, hyphae from zones A, B, respectively, as seen in longitudinal section ($\times 500$). *Pralliota arenaria*. (g, diagram to show arrangement in transverse section: A, central zone of narrow hyphae; B, cortical zone of narrow hyphae; C, peripheral mantle of loosely arranged narrow hyphae. (h, to show arrangement in longitudinal section, lettering as in (g). (i-k, hyphae from zones A-C, respectively, as seen in transverse section ($\times 500$). (l-n, hyphae from zones A-C, respectively, as seen in longitudinal section ($\times 500$).

defined zones, of which the inner one consists of large elements with few air spaces and the outer is of smaller loosely arranged hyphae. In all these three species both zones contribute to the formation of lateral branches.

Rhizomorphs of the two species of *Psalliota* studied resemble one another and have three fairly well-defined zones (Fig. 2*g-n*). The 'cortex' of narrow hyphae is differentiated into an inner closely woven and an outer loosely constructed zone. All three zones take part in branch formation.

None of the above five species shows a firm sheath to the rhizomorph such as that of *Hymenogaster tener*.

The rhizomorphs of *Hysterangium nephriticum* and *Phallus impudicus* differ from all others studied in having a compact inner core of narrow hyphae running mainly in a longitudinal direction and an outer zone of wider hyphae (Fig. 3*a-e*). The latter are derived from the margin of the core, the hyphae of which turn outwards to grow in a radial direction, become inflated and by septation produce more or less globose cells. In *Hysterangium* these remain almost entirely free from one another, but in *Phallus* they fuse and by division in more than one plane give rise to a compact pseudoparenchyma (Fig. 3*e*). In both species both zones participate in branch formation and the outer zone is continuous with the peridia of the fruit-bodies, which develop terminally on the rhizomorphs, while the more compact inner zone differentiates to form the gleba.

Rehsteiner (1892) and Fitzpatrick (1913) considered that the Phallaceae are derived from the Hysterangiaceae. It is thus of interest that the rhizomorphs of *Hysterangium nephriticum* and *Phallus impudicus* are so strikingly similar and unlike those of any other species studied. Too much significance should not be given to this fact since the rhizomorph of *Mutinus* is relatively simple and shows no close similarity to that of the nearly related *Phallus*.

(c) *Zonation due to differences in arrangement, size and structure of hyphae.* In the rhizomorphs described above, the constituent hyphae are thin-walled even when they vary in width and arrangement. A further development shown by *Lycoperdon pyriforme*, *L. gemmatum*, *Marasmius androsaceus* and *Armillaria mellea* is the thickening of the cell walls of some of the hyphae.

Rhizomorphs of *Lycoperdon pyriforme* and *L. gemmatum* consist of three zones of hyphae (Fig. 3*f*). The central core is composed of thick-walled hyphae, which vary in diameter from 5 to 10 μ and increase generally in size towards the centre, interspersed with narrow thin-walled hyphae (diameter 3 μ). The proportion of the latter increases towards the outer edge of this central zone (Fig. 3*g*). The cells of the large thick-walled hyphae are separated by oblique or transverse septa which often show clamp connexions. The central core is surrounded by a thin cylinder of fine interwoven hyphae (diameter 3 μ) and sharply marked off from the outer zone of loosely interwoven elements (Fig. 3*g, h*). In *L. gemmatum* this outer layer is wider and more diffuse than in *L. pyriforme*. Rhizomorphs of the latter species occurring within decaying bark or wood differ from those grown in soil in the greater number and diameter of the thick-walled hyphae of the core.

Lateral branches in both species arise from the middle zone of fine compact hyphae, and the loose peripheral zone of the branch is continuous with that of the main strand. A few thickened hyphae are present at the

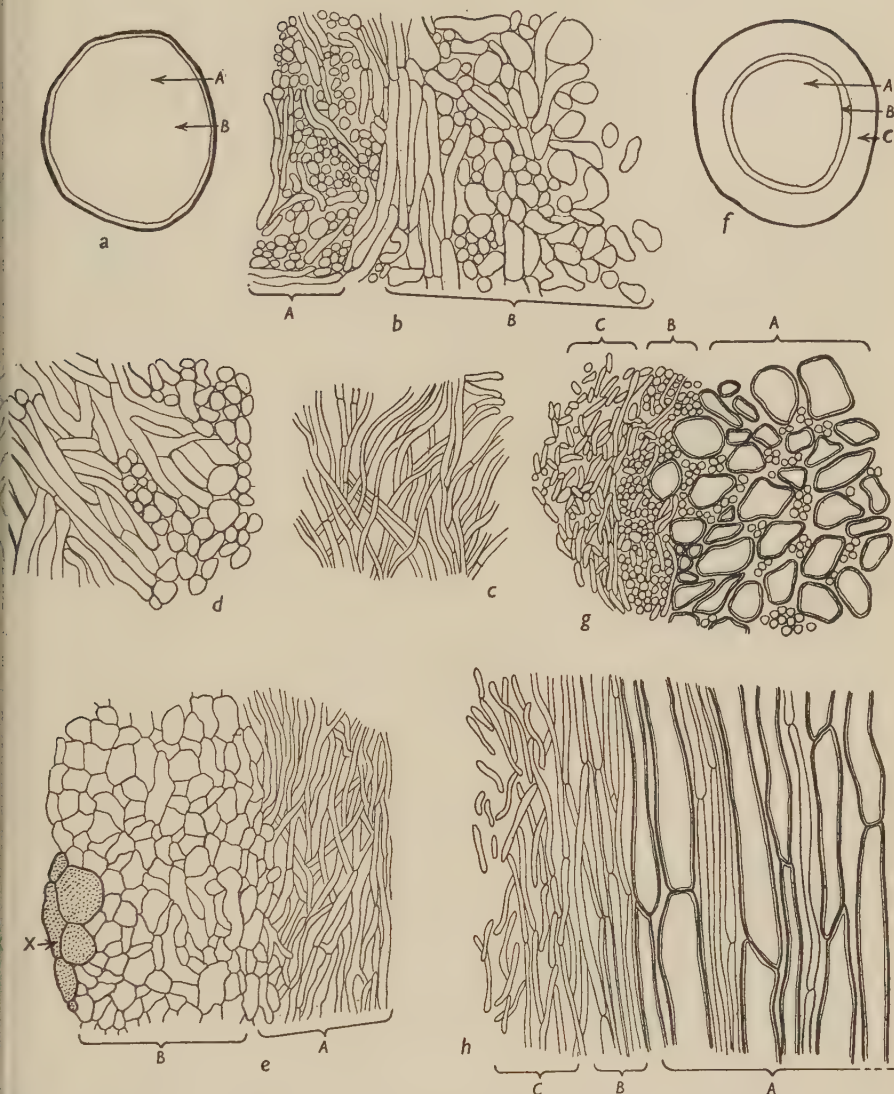


Fig. 3. *Hysterangium nephriticum*. (a) diagram to show arrangement as seen in transverse section: A, central zone of narrow hyphae running more or less parallel to axis of rhizomorph; B, narrow outer layer of broad hyphae which by septation form a firm margin. (b) hyphae from central zone (A), and peripheral zone (B), in transverse section ($\times 500$). (c, d) hyphae from central (A) and peripheral (B) zones in longitudinal section ($\times 500$). *Phallus impudicus*. (e) hyphae from longitudinal section of rhizomorph: A, narrow hyphae from central core; B, pseudoparenchyma of septate and inflated hyphae; X, soil particles which adhere closely to outer hyphae or may become enclosed. *Lycoperdon pyriforme*. (f) diagram to show arrangement in transverse section: A, central core of broad, thick-walled hyphae mixed with narrow thin-walled ones; B, narrow zone of fine hyphae, compactly arranged; C, marginal zone of fine hyphae, loosely arranged. (g) hyphae from zones A-C, in transverse section ($\times 500$). (h) hyphae from zones A-C, in longitudinal section ($\times 500$).

base of the branch, and typical thick-walled elements develop as the branch grows.

Splitting of the rhizomorphs of *L. pyriforme* frequently occurs, resulting in the formation of loops. A crack develops in the central zone and widens until it extends for the whole width of the strand. The inner side where the broad cells are exposed soon becomes covered with a thin crust of narrow hyphae.

In contrast to *Hysterangium nephriticum* and *Phallus impudicus* the zones of the rhizomorphs of *Lycoperdon* spp. do not correspond to definite parts of the fruit-bodies, and a rhizomorph which is about to give rise to an apical fruit-body loses its characteristic zonation so that the region just below the developing puff-ball is a homogeneous mass of interwoven hyphae running more or less in the direction of the axis of the strand.

Swartz (1933, 1936) showed that the rhizomorphs of *Lycoperdon pedicellatum* are essentially similar to those of *L. pyriforme*, while those of the related *Calvatia saccata* and *Bovista plumbea* are rather less complex.

While the rhizomorphs of the Lycoperdaceae show a thickening of the walls of the individual conducting hyphae, those of *Marasmius androsaceus* and *Armillaria mellea* develop an outer rind or crust of fused thick-walled hyphae which protects the inner conducting area from desiccation. In both species this rind is formed from cells which are irregular in both size and shape, closely packed so that there are no intercellular spaces, and with dark brown or black walls so thickened and fused that, in transverse section, individual cells can only be distinguished by their narrow lumina, the original cell-walls being indiscernible.

In *Marasmius androsaceus* (Fig. 4*a-e*) the cells of the rind are never more than 5μ in diameter and are of $140-180\mu$ long (Fig. 4*b, c*). Within the rind the core consists of irregularly shaped cells with dense contents. These cells, which are $4-8\mu$ wide and $20-50\mu$ long are compactly arranged immediately within this rind, and are more loosely interwoven to form a network at the centre.

This agrees with Macdonald's (1949) description. In a young rhizomorph the rind is much narrower and is surrounded by a narrow layer of thin-walled hyphae. Later these marginal hyphae become thickened and are indistinguishable from the rest of the rind. The medulla of a young rhizomorph is homogeneous in arrangement and has no intercellular spaces. These develop later as the rhizomorph increases in width. Growth thus involves the progressive thickening of the walls of the outer hyphae and the expansion of the medulla by the separation of the hyphae which, as also observed by Macdonald (1949), are originally twisted round one another.

Lateral branches arise from the outer medulla (Fig. 4*d*) and both medulla and rind of the branch are continuous with those of the parent rhizomorph.

Armillaria mellea has two types of rhizomorphs: hard brown or black, cylindrical or flattened strands, formed in the soil or on or near the surface of decaying tree trunks or branches; softer white, flattened strands, formed within the wood of infected trees or deep in the medium of agar cultures. Earlier investigators, such as Nees v. Esenbeck, believed that

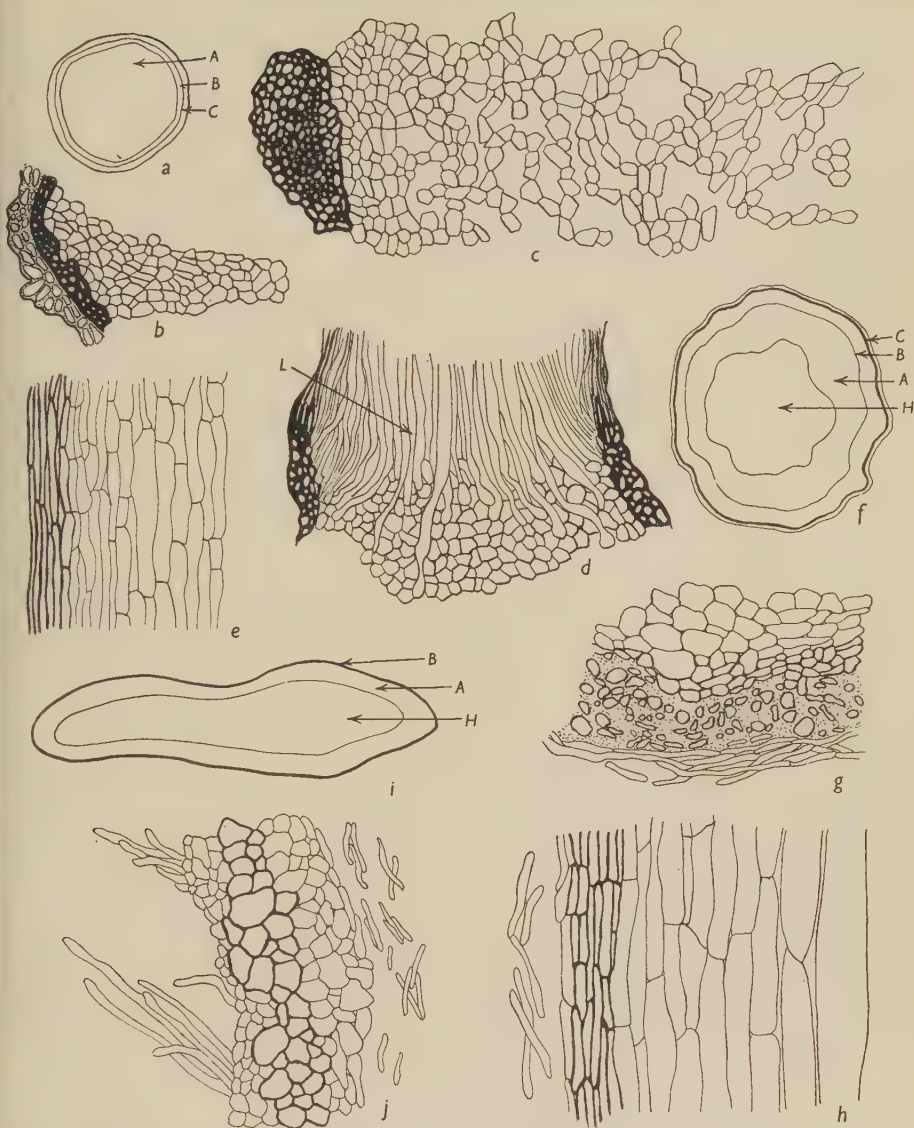


Fig. 4. *Marasmius androsaceus*. (a) diagram to show arrangement of hyphae of young rhizomorph, as seen in transverse section: A, central of thin-walled cells; B, rind of thick-walled, fused cells; C, outermost layer of cells with less strongly thickened walls. (b) hyphae from zones A-C, of young rhizomorph in transverse section ($\times 500$). (c) hyphae from mature rhizomorph in transverse section. The marginal zone (C) is no longer distinguishable, and the rind (B) has increased in width ($\times 500$). (d) transverse section through base of lateral branch (L) ($\times 500$). (e) hyphae of rind (B) and core (A) of mature rhizomorph, seen in longitudinal section ($\times 500$). *Armillaria mellea*. (f) diagram to show arrangement of hyphae in *Rhizomorpha subterranea* type, as seen in transverse section: A, zone of broad thin-walled cells; B, rind of thick-walled, fused cells; C, outer layer of filamentous, thin-walled hyphae; H, hollow centre. (g), hyphae from the three zones in transverse section ($\times 500$). (h) hyphae from the three zones in longitudinal section ($\times 500$). (i) diagram to show arrangement of hyphae in *R. subcorticalis* type, as seen in transverse section: H, hollow centre; A, zone of broad, thin-walled cells; B, zone of cells with slightly thickened walls ($\times 500$). (j) *R. subcorticalis* in transverse section, showing inner zone of loose hyphae extending into hollow centre surrounded by more compact zone including some hyphae with thickened walls and marginal layer of thin-walled cells, some of which extend outwards.

each type of rhizomorph represented a distinct species of fungus and the two types were termed *Rhizomorpha subterranea* and *R. subcorticalis*, respectively. The relationship between these and the fruit-bodies was finally elucidated by Hartig (1873). De Bary (1884) considered that the form of the rhizomorph is determined by nutritional factors. Hamada (1940) showed that on yeast agar the *R. subterranea* type of rhizomorph predominated while the *R. subcorticalis* type was produced on soya sauce agar. He suggested that undetermined growth substances in these media were the factors determining rhizomorph form.

The *subterranea* type (Fig. 4e-g) is similar in structure to the rhizomorph of *Marasmius androsaceus*. The margin of young rhizomorphs consists of narrow hyphae ($2-3\mu$ diameter), some of which project outwards to give an irregular mantle surrounding the developing rind. In older ones this mantle is absent and the pseudosclerenchymatous rind resembles that of *M. androsaceus*. It consists of thick-walled hyphae $4-7\mu$ wide, more or less elongated longitudinally but more frequently septate on the outer side of the rind. The central medulla or core is a region of thin-walled hyphae, the cells of which are large in the central region (outer elements $5-7 \times 30-50\mu$, inner elements $12-15 \times 135-140\mu$). In mature rhizomorphs the inner medullary cells tear apart or break down to leave a hollow centre.

The apex of a mature rhizomorph consists of thickly interwoven hyphae surrounding a core of even denser material. The mantle of loose hyphae extends from a point 5 mm. behind the tip to a point about 10 mm. from the apex, where both differentiation of the rind and the production of lateral branches begins.

The first sign of branch formation is the abundant growth of the outer filamentous mantle which probably serves to protect the growing apex of the young branch. The cells of the outer medulla multiply and this is followed by proliferation of the cells of the rind which is thus caused to bulge outwards.

The *subcorticalis* type is more uniform in structure than *Rhizomorpha subterranea*. All the cells are more or less circular in cross-section and vary in diameter from 3 to 8μ . The central region is hollow with a few scattered hyphae. Some of the cell-walls in the outer region are slightly thickened but are not dark coloured. The extreme margin consists of an irregular layer of cells with a few projecting hyphae (Fig. 4h, i).

FACTORS INFLUENCING THE DEVELOPMENT OF RHIZOMORPHS OF *ARMILLARIA MELLEAE* IN CULTURE

Reitsma (1932) and Hamada (1940) have studied some of the factors influencing the growth of rhizomorphs of *A. mellea*, and recently Garrett (1953) has investigated the effect of nutrition on the initiation of the rhizomorphs.

While the actual numbers of initials developing can be counted, there is no simple and generally applicable method of estimating further development. In the present experiments the average diameter of the area penetrated by the rhizomorphs of a colony usually gave satisfactory results if the number of replicates was large. It was unsuitable where the conditions to be compared produced few long rhizomorphs in contrast to

numerous short ones or curled ones in contrast to straight ones. It also gave no measurement of the degree of branching. The best method of expressing rhizomorph growth was as rate of increase in total length, obtained by tracing the actual rhizomorphs or, less laboriously but also less accurately, by tracing the outline of the colony and estimating the distance from the inoculum to the tip of each main rhizomorph.

Temperature. Four plates of 3 % malt agar, inoculated with mycelium of *A. mellea*, were incubated at each of the following temperatures 10, 20, 25, 30 and 37° C.

After 2 days' incubation, the cultures at 25 and 20° C. showed signs of mycelial growth. Those at 30 and 10° C. did not begin to grow until the fourth and the sixth day, respectively.

Rhizomorphs began to develop at 20 and 25° C. on the fourth day. They grew rapidly in a radial direction and had reached the margin of the Petri dish by the end of a fortnight. At 30° C. they grew very slowly and were only 2 mm. after a week's incubation. None were produced on the cultures at 10° C. and no growth took place at 37° C.

The cultures at 20 and 25° C. produced colonies of similar extent, but when the total rhizomorph lengths of the colonies were estimated, those at 20° C. averaged 210 mm./colony, whilst those at 25° C. averaged 236 mm./colony. Thus, although the rhizomorphs had penetrated areas of similar dimensions at these two temperatures, the production of lateral branches was abundant at 25° C., whereas few were produced at 20° C.

At the end of a fortnight, the plates at 20 and 25° C. were entirely filled but the colonies extended to a distance of only one-quarter of the diameter of the plate at 30° C.

At this temperature, however, the rhizomorphs were numerous and were produced in a dense cluster. The differences in the rate of growth of rhizomorphs at 20 and 25° C. were now more obvious, those at 25° C. being much more vigorous.

Reitsma (1932) reports that 25° C. is the optimum temperature for growth of *A. mellea*. While linear growth of mycelium was equally rapid at 20° C., in the present experiment both mycelium and rhizomorph development was more vigorous at 25° C. All subsequent experiments were therefore incubated at 25° C. unless otherwise stated.

Humidity. Plates of 3 % malt agar were inoculated with mycelium of *A. mellea*, and after 2 days were inverted and received the following treatments (in triplicate): (a) sterile water was poured into the inverted lids; (b) dilute H_2SO_4 was poured into the inverted lids (1/5; 89 % relative humidity); (c) conc. H_2SO_4 was poured into the inverted lids; (d) control, no additions.

There was no further growth or rhizomorph production in cultures that were incubated over sulphuric acid. On the media that were incubated over water, the production of rhizomorphs was much greater than on the control cultures. On the latter there was a plentiful development of brown pseudosclerenchymatous membranes on the surface of the media, but on the former the media were covered by white mycelium.

This experiment indicates that the production of rhizomorphs is

encouraged by moist conditions, but that excessive humidity prevents the formation of pseudosclerenchyma.

Light. Total length of rhizomorphs and diameters of colonies were greater in plates of 3% malt agar wrapped in black paper than in unwrapped ones placed beside them on a well-lighted window sill. The number of rhizomorphs produced did not differ significantly. Thus while light does not inhibit the formation of rhizomorph initials it slows down the elongation of the rhizomorphs.

H-ion concentration. Tartaric acid or sodium carbonate was added to a glucose-peptone medium to give a range of H-ion concentration from 1.6 to 9.2. Growth took place over a range of initial pH 3.1–8.8 but rhizomorphs were formed only over a slightly narrower range, pH 3.6–8.0. The optimum initial pH was 5.6 (thus agreeing well with Benton & Ehrlich's (1941) estimate of 5.0) but, as is usual with the effect of H-ion concentration on fungal growth (Hawker, 1950), this optimum was not clearly defined and growth was almost equally good on either side of pH 5–6.

The effect of the ratio of carbon to nitrogen. The ratio of carbon to nitrogen has been shown to have a profound effect on the formation of sclerotia of certain fungi (Peiris, 1947; Garrett, 1949; Townsend, 1952). Garrett (1951) considers that rhizomorphs and sclerotia show morphological, physiological and even functional similarities. Accordingly, the effect of the carbon-nitrogen ratio on rhizomorph initiation and development was studied. Since this work was completed Garrett (1953) has published the results of similar experiments. A detailed account of the present experiments will not therefore be given here, as they have already been recorded (Townsend, 1952).

To a basic medium containing potassium dihydrogen phosphate and magnesium sulphate, glucose was added to give concentrations of 0.0–3.0% and either peptone or asparagine to give concentrations of 0.0–1.0%.

The amount of glucose required for good growth of rhizomorphs was higher than that of the nitrogen substance. Excess or absence of either carbon or nitrogen inhibited rhizomorph initiation. A ratio of three to five parts of glucose to one of peptone or asparagine was the most favourable. The amount of glucose optimal for the initiation and development of rhizomorphs increased with increase in nitrogen content of the medium. Similarly, the optimal concentration of nitrogen increased with increased amounts of glucose.

None of the other species examined formed rhizomorphs under the conditions of these experiments. It is clear that culture conditions bear little relation to those obtaining in the soil or in decaying wood, but results obtained in artificial culture may give some guide to the interpretation of the complex natural environment.

Thanks are due to Dr L. E. Hawker for suggesting and directing this study, and for assistance in the preparation of the manuscript.

REFERENCES

- BENTON, V. L. & EHRLICH, J. (1941). Variation in culture of several isolates of *Armillaria mellea* from Western White Pine. *Phytopathology*, **31**, 803-811.
- DE BARY, A. (1884). *Comparative morphology of the Fungi, Mycetozoa and Bacteria*. Oxford.
- FITZPATRICK, H. M. (1913). A comparative study of the development of the fruit body in *Phallo-gaster*, *Hysterangium* and *Gautieria*. *Ann. mycol., Berl.*, **11**, 119-149.
- GARRETT, S. D. (1944). *Root disease fungi*. Waltham, Mass., U.S.A.
- GARRETT, S. D. (1949). A study of violet root rot. II. Effect of substratum on survival of *Helicobasidium purpureum* colonies in the soil. *Trans. Brit. mycol. Soc.* **32**, 217-223.
- GARRETT, S. D. (1951). Ecological groups of soil fungi. *New Phytol.* **50**, 149-166.
- GARRETT, S. D. (1953). Rhizomorph behaviour in *Armillaria mellea* (Vahl) Quél.
I. Factors controlling rhizomorph initiation by *A. mellea* in pure culture. *Ann. Bot., Lond.*, **17**, 63-79.
- HAMADA, M. (1940). Physiologisch-morphologische studien über *Armillaria mellea* (Vahl) Quél., mit besonderer Rücksicht auf die Oxalsäurebildung. *Jap. J. Bot.* **10**, 387-464.
- HARTIG, R. (1873). Vorläufige Mitteilung über den Parasitismus von *Agaricus melleus* und dessen Rhizomorphen. *Bot. Ztg.*, **31**, 295-297.
- HAWKER, L. E. (1950). *Physiology of fungi*. London.
- MACDONALD, J. A. (1949). The heather rhizomorph fungus, *Marasmius androsaceus* Fries. *Proc. Roy. Soc. Edinb. B*, **43**, 230-241.
- PEIRIS, J. W. L. (1947). The *Botrytis* disease of *Gladiolus*, together with a physiological study of certain *Botrytis* species. Thesis for the Degree of Ph.D. in the University of London.
- REHSTEINER, H. (1892). Beiträge Zur Entwicklungsgeshichte der Fructkörper einiger Gastromyceten. *Bot. Ztg.*, **50**, 777-792.
- REITSMA, J. (1932). Studien über *Armillaria mellea* (Vahl) Quél. *Phytopath. Z.* **4**, 461-522.
- TOWNSEND, B. B. (1952). The morphology and physiology of the sclerotia and rhizomorphs of certain fungi. Thesis for the Degree of Ph.D. in the University of Bristol.
- SWARTZ, D. (1933). Some developmental characters of species of Lycoperdaceae. *Amer. J. Bot.* **20**, 440-463.
- SWARTZ, D. (1936). The development of *Lycoperdon pedicellatum*. *Amer. J. Bot.* **23**, 4-7.

(Accepted for publication 31 July 1953)

CANDELABRUM SPINULOSUM: A NEW BRITISH RECORD

By T. L. C. BOTTOMLEY

23 Ryegate Crescent, Sheffield 10

(With 1 Text-figure)

The aero-aquatic Hyphomycete, which is the subject of this record, was found on decaying leaves of beech and oak from the bottom of old mill dams in Whiteley Woods and Rivelin Valley, Sheffield, during May and June 1953. These dams are fed by small streams and in most there is a large accumulation of decaying leaves, the lower layers of which have a characteristic black appearance, probably due to more or less anaerobic conditions.

It is upon these leaves that this fungus is found.

The mycelium grows on the surface of the leaves and is hyaline, branched and septate, the individual hyphae are about 3μ wide. When the leaves are exposed to the air for a few days in a covered dish, the conidiophores project at right angles to the mycelium and grow through the film of water on the leaf surface and produce conidia in the air. The conidiophores are variable in length, ten measured in lactophenol were $20-50\mu$ long by $2-3\mu$ wide, the apex tapers to rather less than 1μ at the point of attachment of the conidium.

The conidia appear as minute white rings when viewed under a magnification of $\times 60$, but are very striking when seen under high magnification ($\times 1000$). When viewed from the top there is a ring of eight more or less rounded cells covered with blunt spines (Fig. 1*a*) and with an H-shaped body of four smooth cells in the centre, two of which are large and two small (Fig. 1*c*). In side view, the eight cells are seen to be irregularly oblong in shape, there being two lateral cells growing from each cell of the central body (Fig. 1*b*). The conidia sometimes have a short 'stalk' $2-3 \times 1\mu$ attached to one of the central cells (Fig. 1*d*). Conidia germinate readily in distilled water, each lateral cell sending out one or two germ tubes which reach a length of from 50 to 80μ in 24 hr. (Fig. 1*e*). The spines do not stain in Cotton Blue. The conidial measurements (ten conidia measured in lactophenol) are: diameter in top view $13-15\mu$; in side view $13-15 \times 10\mu$; diameter of apex of lateral cells 3μ ; single lateral cell $10 \times 2.5-4\mu$; central body of four cells $10 \times 10\mu$.

Pure cultures were established on 2 % malt agar from conidia picked off leaves with a fine glass needle. Growth was rather slow, cultures grew to about 2 cm. in diameter in 4 weeks in daylight. The central part of the culture was cream coloured and rather woolly in appearance, being formed of vegetative hyphae growing together in tufts. No spores were observed on this portion. The outer part of the culture was white and felty in appearance and formed spores abundantly, and at the edge of the culture

there was a narrow band, 1–2 mm. wide, of what appeared to be submerged mycelium in the medium. When a culture, or a leaf upon which the fungus is growing, is flooded with water, the mature conidia rise to the surface because of an air bubble which is trapped between the projecting cells of the conidium.

This interesting little fungus was described as the type species of a new genus with the name of *Candelabrum spinulosum* (van Beverwijk, 1951; syn.

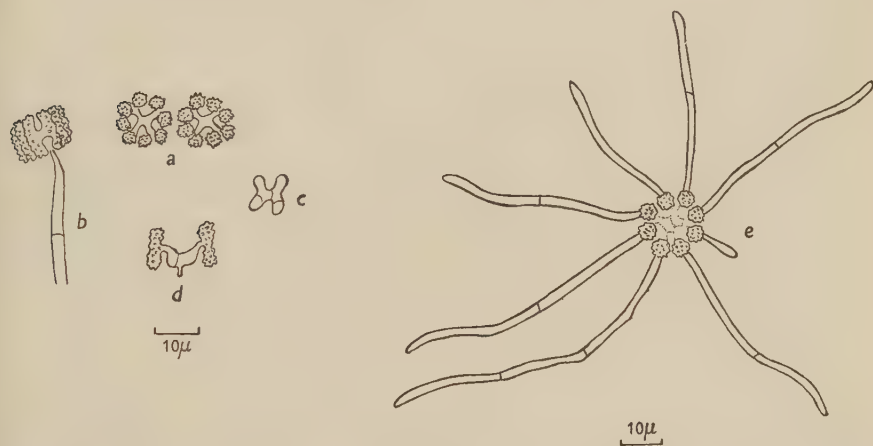


Fig. 1. *a*, conidium seen from above, top of lateral cells in focus; *b*, conidium in side view showing lateral cells and conidiophore; *c*, the four central cells; *d*, optical section of side view showing attachment 'stalk'; *e*, germinating conidium, each cell has sent out one germ tube. (All $\times 500$.)

Dyonisia coronata Arnaud, 1952) in Holland, Sweden and Switzerland. A culture of this fungus has been sent to Miss van Beverwijk, who kindly identified it. A slide has been deposited in the Herbarium C.M.I. under No. 53086.

I would like to express my thanks to Miss A. L. van Beverwijk for her kind help, and also to Mr John Webster, of the University, Sheffield, for his help and advice on many occasions.

REFERENCES

- ARNAUD, G. (1952). Mycologia concrete: genera. *Bull. Soc. mycol. Fr.* **68**, 181–223.
 VAN BEVERWIJK, A. L. (1951). *Candelabrum spinulosum* a new fungus species. *Leeuwenhoek ned. Tijdschr.* **17**, 6–12.

(Accepted for publication 4 August 1953)

STUDIES IN THE MYRIANGIALES

IV. *SPHACELOMA HESPERETHUSAE* SP.NOV. ON
HESPERETHUSA AND *PLEIOSPERMIUM**

BY ANNA E. JENKINS

Foreign Agricultural Service, U.S. Department of Agriculture

AND A. A. BITANCOURT

Instituto Biológico, São Paulo, Brazil

(With Plates 7 and 8)

Sphaceloma hesperethusae sp.nov. is described on fruit and leaves of *Hesperethusa crenulata* (Roxb.) Roem. from India, and, provisionally, on fruit of two other closely related citrus relatives, viz. *Pleiospermium alatum* (Wt. & Arn.) Swing., India (Eastern Ghats, near Penagaluru), and *P. sumatranum*, Sumatra. The type specimen consists of the fungus on abundantly scabbed fruit of *hesperethusa* from the Calcutta Botanic Garden (1915). An exceptionally early record of the fungus, or disease, is afforded by the abundantly scabbed fruits of the type specimen of *Hesperethusa crenulata* (*Limonia crenulata* Roxb., 1795).

Swingle (1948),* in alluding to a *Sphaceloma* scab disease of *hesperethusa* (*Hesperethusa crenulata*) (Jenkins, 1936) and the related Sumatra orangeaster (*Pleiospermium sumatranum*) (Bitancourt & Jenkins, 1938; also cf. p. 238), did so under the perfect stage name of this form genus, i.e. *Elsinoë*. We are here returning to the conidial stage name in describing the species on *hesperethusa* as *Sphaceloma hesperethusae*. A scab disease of Ceylon orangeaster (*Pleiospermium alatum*) and that of Sumatra orangeaster are attributed provisionally to the new species.

***Sphaceloma hesperethusae* sp.nov.**

Maculis fructigenis sparsis vel in aream irregularem coalescentibus, saepe ultra tertiam partem fructus sicci tegentibus, singulus maculis quasi circularibus, raro irregularibus, 0.2–0.6 mm., interdum usque 1.5 mm. latis, margine bene limitato, saepius surrecto, pulvinato, aut frequenter omnino convexo et bulloso, in medio laete cinnamomeo, margine pallidiore. Maculis in centrali parte saepe applanatis vel leviter depressis necnon obscure coloratis ob praesentiam fructificationum *Sphacelomae*.

Acervulis vel sporodochiis stroma vulgo continuum in centrali macularum parte in margine 40–50 μ crassum formantibus. Stromate homoganeo pseudo-parenchymatoso, e cellulis circiter isodiametralibus, irregularibus, circiter 5 μ longis, parietibus crassis et obscuris compositis, sed in mediis partibus stromatis gradualiter ad plectenchyma pallidum transeunte. *Conidiis* minime visis.

Hab. Indiis orientalibus ad fructus *Hesperethusae crenulatae* et *Pleiospermi alati*, et in Insula Sumatra ad fructus *Pleiospermi sumatrani*.

Spots on the dry fruit, scattered or coalescing into an irregular area, sometimes covering more than one-third of the surface, individually round or nearly so, rarely irregular, 0.2–0.6 mm. in diameter, occasionally up to 1.5 mm. with sharply delimited, often raised margin, pulvinate, sometimes

* In this article the classification of Rutaceae (Aurantioideae) follows Swingle as just cited.

very prominent and knoblike, central area 'cinnamon buff',* periphery paler, fertile central part of lesion often flattened or slightly depressed as well as darkened by the superficial growth of the *Sphaceloma*. *Acervuli*, or sporodochia, usually forming a more or less continuous stroma in the central area of the spots, reaching 40–50 μ in thickness, in the outer layers composed of a homogeneous pseudo-parenchyma with nearly isodiametric irregular cells, about 5 μ in diameter, and with thick, dark walls, this tissue gradually transforming into a pale plectenchyma in the inner layers. *Conidia* not seen (Pl. 7, figs. 6–8).

Habitat. Producing a scab on rind of fruit, and on peduncles and leaves of *Hesperethusa crenulata* (Roxb.) Roem. (subtribe Citrinae, subfamily Aurantioideae, family Rutaceae), India: Calcutta Botanic Garden, near Sibpur, and Coromandel Coast, including forests of the Eastern Ghats near Penagaluru (elevation, 300–1000 m.), Cuddapah (DT).

Type—interception, Washington, D.C., 15 February 1916, from ripe fruit gathered in the Calcutta Botanic Garden, December 1915, and presented by the Curator to W. T. Swingle—in Mycol. Coll., U.S. Dep. Agric. 68712–13; Herb. I.M.I., Kew, England; Herb. Kew. (Tanaka, Q 1258, examined 1928), Kew; Herb. Inst. Biol. 2073; also distributed in Jenkins and Bitancourt's *Myriangiales selecti exsiccati* 469.

Attributed provisionally to *Sphaceloma hesperethusae* are the scab diseases affecting two other species in the subtribal group 'Primitive Citrus Fruit Trees' of the Citrinae, namely, *Pleiospermium alatum* (Wt. & Arn.) Swing., Eastern Ghats near Penagaluru, and *P. sumatranum* Swing., Sumatra.

The records of *Sphaceloma hesperethusae* on wild hesperethusa growing on the Coromandel Coast are based in part on the presence of typical scab lesions on phanerogamic herbarium specimens in the Herb. Kew. Thus, in 1937, Dr S. F. Ashby, then director of the Commonwealth Mycological Institute, kindly examined the specimens of *Hesperethusa crenulata* from India for scab lesions corresponding to those on the fruits from the Calcutta Botanic Garden as illustrated by Jenkins (1936, Fig. A, B). Such lesions, apparently, were present on fruit of the type specimen of *Limonia crenulata* (= *Hesperethusa crenulata*), described from the Coromandel Coast (Roxburgh, 1795). Dr Ashby therefore requested samples for comparison. His letter† acknowledging the receipt of 'three fruits of *Hesperethusa crenulata* showing clearly the characteristics of the *Sphaceloma* scab lesions' also reported his findings as follows:

With the kind consent of Mr A. D. Cotton, Keeper of the Herbarium, Royal Botanic Gardens, I have sent you, under another cover, two fruits out of the twelve in a packet attached to the sheet of Roxburgh's type of *L. crenulata*; about half the fruits in the packet showed scab lesions. On the two twigs with leaves no scabs seemed to be present. These specimens on half the sheet were from the Forster Herbarium. On the other half of the sheet were specimens from Herb. Rottlerianum, Penins., Indiae Orientalis. One of these bears three fruits each with a number of quite similar scab lesions; the twig and leaves of these specimens also seem free from scab.... I also looked at a folder of specimens of *H. crenulata* from Siam but there was nothing resembling the scab on them.

The close correspondence of the scab lesions on the two fruits from Roxburgh's type (Pl. 7, figs. 1, 3 and 4) to those on the interception from

* Colour readings in quotations are based on Ridgway (1912).

† Letter to A. E. J. dated 23 September 1937.

the Calcutta Botanic Garden (Pl. 7, fig. 2, and Jenkins, 1936, fig. 1 B) is shown by the illustrations just cited. A slight and probably insignificant difference is the somewhat less regular outline of the lesions on the specimen from the Coromandel Coast. At Dr Swingle's request one of these fruits was contributed toward his further botanical study of the monotypic genus *Hesperethusa*. The other fruit is accessioned under *Sphaceloma hesperethusae* in the U.S. Mycological Collections (74464). Roxburgh's phanerogamic specimen, placed early in the chronicle of Indian botany,* now stands as the earliest known specimen of any species of *Sphaceloma* or of *Elsinoë*.

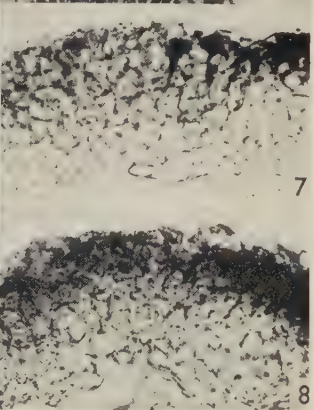
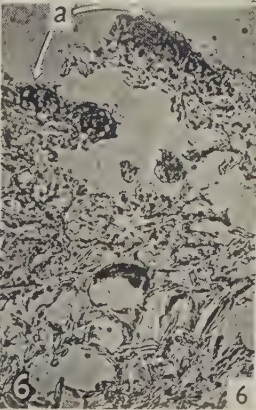
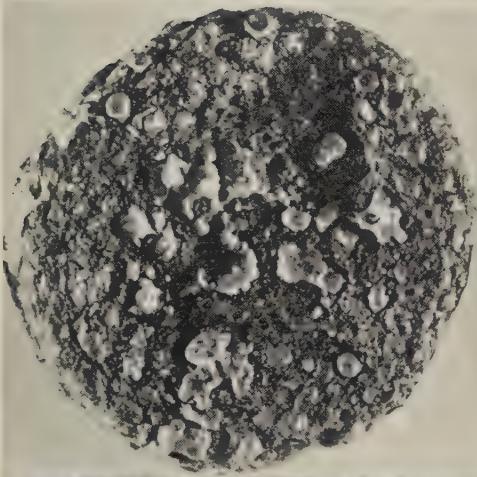
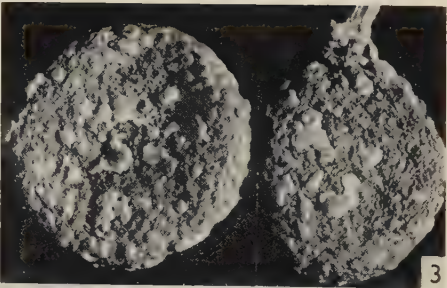
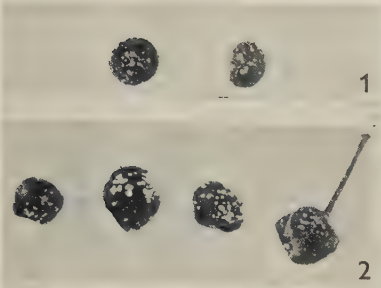
For a second as well as a comparatively recent specimen of *Sphaceloma hesperethusae* on *hesperethusa* from the Coromandel Coast, more specifically from the Eastern Ghats near Penagaluru, we are indebted to Prof. D. Raghava Reddy of that place. His several excursions to search for scab diseases of wild relatives of citrus growing naturally in that forest region purposely were made late in the year, when the fruit would have ripened. *Hesperethusa* scab, as he discovered it in 1946, affected leaves only of that Citrinae, and many of the lesions seemed to have followed mechanical injury, perhaps fresh spine pricks (Pl. 7, fig. 5). (Specimen preserved in Herb. I.B. 6016, U.S. Mycol. Coll. 91244.)

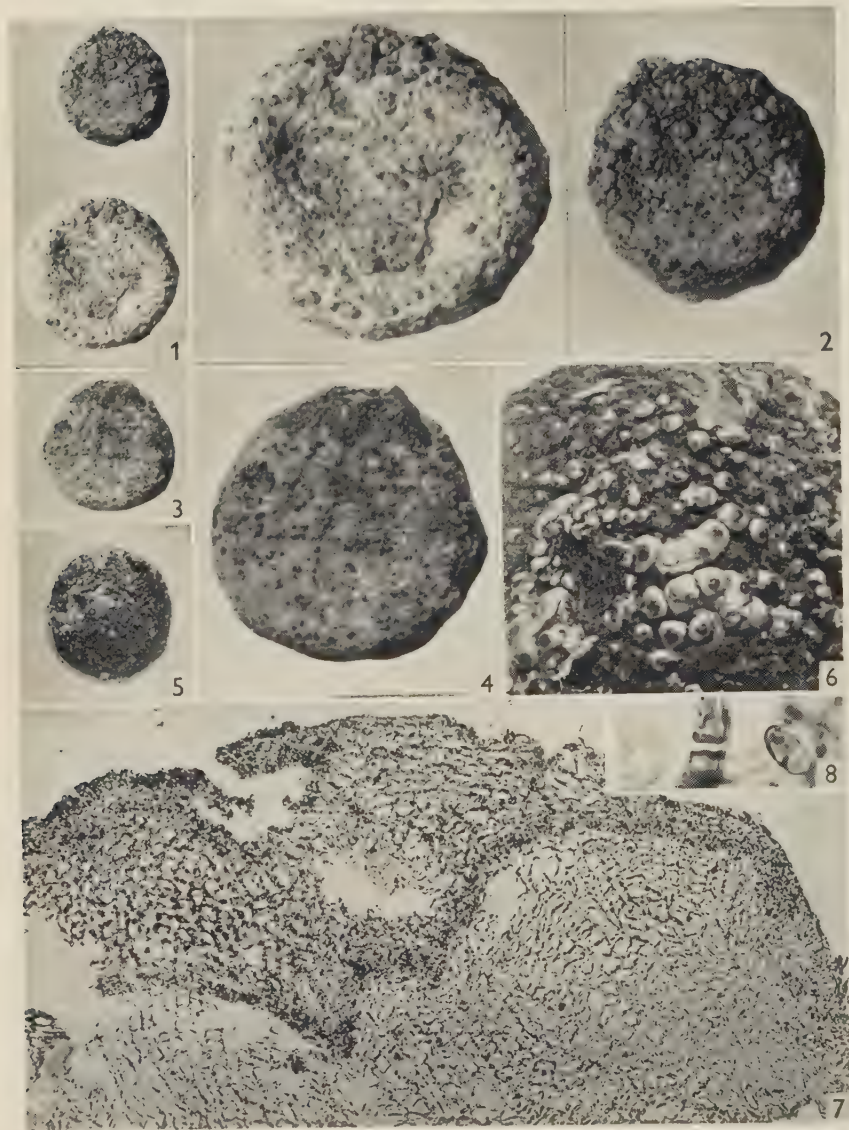
Ceylon orangeaster scab was discovered by Prof. Reddy on an earlier excursion to the Eastern Ghats near Penagaluru, in December 1939. In this case only fruit lesions were found, and these were exceedingly numerous as well as usually disposed over the oil glands of the rind. They resemble the fruit lesions of *hesperethusa* scab, except that here the darkened central area arises from the visibility of the dark oil glands through the transparent lesion tissue (Pl. 8, figs. 5, 6). The specimen of scabbed fruits contributed by Prof. Reddy has been divided and distributed to several different herbaria (U.S. Mycol. Coll. 90081, Herb. I.B. 5633, Herb. I.M.I., and Herb. Kew). These typical *Sphaceloma* scab lesions are sterile in so far as we have been able to discern.

According to Swingle (1948) *Pleiospermium sumatranum* is 'known only from Korthals' collections, probably from a single locality [in Sumatra]'. Our report (1938) of *Sphaceloma* on this orangeaster emanated from Dr Swingle's having borrowed these rare phanerogamic collections from the Rijksherbarium for his own taxonomic need, then incidentally having observed and called our attention to the abundantly scabbed fruit. The excrescences are clearly apparent on photographs of the specimens taken before they were returned.† From that source, in addition to quoting from Swingle (1939) for citation of the specimens, we are here illustrating two fruits from 'Korthals S 960 (Type in Rijksherbarium, Leiden, sheet No. "908, 204 - ...137")' (Pl. 8, figs. 1, 2) and a fruit from "Herb.

* In *Current Science*, vol. 6, March 1938, p. 477, we read: 'Wm. Roxburgh who is rightly called "the father of Indian botany" succeeded Col. Kyd, the Hony. Superintendent [of the Royal Botanic Garden, Calcutta], in 1793 as the first official superintendent. Roxburgh's *Flora Indica* and his *Icones*, formed the basis of all subsequent works on Indian Botany.'

† U.S.D.A. Div. of Plant Exploration & Introd., negative No. 84949 (Rijksherb. sheet No. '908, 204 - ...137') and negative No. 84952 (Rijksherb. sheet No. '908, 203 - ...210').





Korthals" in Rijksherbarium, Leiden, sheet No. "908, 203 - ...210" (Pl. 8, figs. 3, 4).

For our microscopic examination Dr Swingle dissected three lesions of our own selection, these from the specimen mounted on sheet No. '908, 203 - ...210'. In this way we viewed sections of the typical *Sphaceloma* scabs (Pl. 8, fig. 7) and determined that minute dark developments rarely present on the surface of the lesions were acervuli of that genus. Associated with them in small number were dusky, elliptical conidia also characteristic of *Sphaceloma*. A guttula is visible toward one end of the example photographed (Pl. 8, fig. 8). An apparently sterile lesion not sectioned is available for reference (Herb. I.B. 2964).

The authors gratefully acknowledge the specimens received through the kindness of the late Dr S. F. Ashby and Mr A. D. Cotton, of Prof. D. Raghava Reddy and the late Dr W. T. Swingle. Thanks also are extended to Rev. D. Bento Pickel for translating the technical description into Latin, as well as to Dr C. O. Erlanson, for contributing the photographs reproduced in Pl. 8, figs. 1-4. Dr Jenkins wishes to acknowledge her association with the United States technical co-operation programme in Brazil, operated jointly by that country and the United States. For the United States the programme is administered by the Technical Co-operation Administration, U.S. Department of State, through the Institute of Inter-American Affairs.

REFERENCES

- BITANCOURT, A. A. & JENKINS, A. E. (1938). New species, hosts, and distribution records of *Elsinoë* and *Sphaceloma*. *Chron. bot.* **4**, 388-389.
 JENKINS, A. E. (1936). A *Sphaceloma* on fruit of *Hesperethusa crenulata*. *Phytopathology*, **26**, 71-73.
 RIDGWAY, R. (1912). *Color standards and color nomenclature*. Washington, D.C.
 ROXBURGH, W. (1795). *Plants of the coast of Coromandel*. ... **1**, 60-61, pl. 86. London.
 SWINGLE, W. T. (1939). *Clymenia* and *Burkillanthus*, new genera; also three new species of *Pleiospermium* (Rutaceae-Aurantioideae). *J. Arnold Arbor.* **20**, 250-263.
 SWINGLE, W. T. (1948). The botany of *Citrus* and its wild relatives of the orange subfamily (Family, Rutaceae; subfamily, Aurantioideae.) In H. J. Webber & L. D. Batchelor, *The Citrus Industry*, **1**, 129-474.

EXPLANATION OF PLATES 7 AND 8

PLATE 7

- Fig. 1. Scabbed fruits from the type of *Hesperethusa crenulata*, Coromandel Coast. $\times 1$.
 Fig. 2. Scabbed fruits of *H. crenulata* from the Calcutta Botanic Gardens. $\times 1$.
 Figs. 3, 4. Enlargements of the fruits in fig. 1. $\times 5$ and $\times 10$, respectively.
 Fig. 5. Part of a leaf of *H. crenulata*, Eastern Ghats, near Penagaluru, showing scab lesions. $\times 4$.
 Figs. 6-8. Conidial stromata of *Sphaceloma hesperethusae* from the type specimen. Fig. 6a, $\times 250$ (after Jenkins, 1937, fig. 1D); figs 7 and 8, $\times 500$.

PLATE 8

- Figs. 1, 2. Scabbed fruits of *Pleiospermium sumatranum*, Korthals S 960 (sheet No. '908, 204 - ...137' in Rijksherb.). $\times 1$ and $\times 2$, respectively.
 Figs. 3, 4. Another scabbed fruit from Korthals's collections in Sumatra (sheet No. '908, 203 - ...210' in Rijksherb.). $\times 1$ and $\times 2$, respectively.
 Figs. 5, 6. Scab lesions on a fruit of *P. alatum*, Eastern Ghats, near Penagaluru. $\times 1$ and $\times 5$, respectively.
 Fig. 7. Section of a lesion on fruit of specimen shown in figs. 3 and 4.
 Fig. 8. Septate hyphal fragment and a conidium of *Sphaceloma* from same source. $\times 1800$. Photographed under oil immersion.

(Accepted for publication 12 October 1953)

AQUATIC PREDACIOUS FUNGI. III

BY MARY PEACH

Biological Laboratories, The Polytechnic, Regent Street, London

(With 6 Text-figures)

Six predacious fungi are described for the first time in this country. They are *Stylopaga rhabdospora* Drechsler, *Zoopage pachyblasta* Drechsler, *Arthrobotrys cladodes* var. *macroides* Drechsler, *A. superba* Corda, *Dactylaria brochopaga* Drechsler, and *Acrostalagmus zeosporus* Drechsler. *Dactylella spermatophaga* Drechsler, which attacks reproductive bodies of pythiaceous fungi, is also described.

During an investigation into the occurrence of predacious fungi in aquatic habitats six species have already been recorded and, of these, two were described as new (Peach, 1948, 1950, 1952). Subsequently two Phycomycetes trapping amoebae, three Hyphomycetes preying on eelworms by means of mycelial traps, and one endozoic Hyphomycete have been identified from samples of soil and water from aquatic habitats. In each case the sample of soil, water or plant debris was used as an inoculum on sterile maize-meal or sterile rabbit-dung agar plates.

***Stylopaga rhabdospora* Drechsler**

This fungus was found in samples from two localities: from the River Thames at Sunbury, Middlesex; and from a stagnant pool at Watermeads, Mitcham, Surrey. The first sample, of leaf debris and mud, was taken 3 in. down and 1 ft. from the bank of the River Thames, but the other, of soil and plant debris, was taken 2 ft. from the edge of the stagnant pool on the settled bank. Sterile rabbit-dung agar was used as a culture medium, and subsequent developments in the two sets of samples were similar.

A phycomycetous fungus was observed which produced delicate aerial conidiophores at intervals over a wide area of the agar surface. Each conidiophore bore a small group of unicellular conidia, the number ranging from three to eight (occasionally nine). The conidia were borne in more or less alternate arrangement (Fig. 1*a*), and on occasion the conidiophore was branched near the base, each branch bearing a terminal group of conidia. Each conidium (Fig. 1*b*) was cylindrical with a sharply pointed proximal end and slightly blunted distal end, and the size range was $16-35 \times 2.2-3 \mu$.

Careful examination showed that the mycelium to which the conidiophores were attached was connected with a haustorium-producing region (Fig. 1*c*). The haustoria were of simple forking type within small amoebae.

Sexual reproduction took place at about 4 weeks from the date of inoculation. Early stages were difficult to observe, but zygosporangia were found which contained zygosporangia with rounded protuberances (Fig. 1*d*). The zygosporangia ranged from 9 to 10 μ in greatest diameter.

This fungus agrees quite well with the original description of *Stylopaga*

rhabdospora given by Drechsler (1936). One point of difference is the common occurrence of more than four conidia in each group in the British material. The smaller number was exceptional in both samples.

Drechsler has also described a strain of *S. rhabdospora* producing larger conidia (1946*a*), but these were not found in the British material.

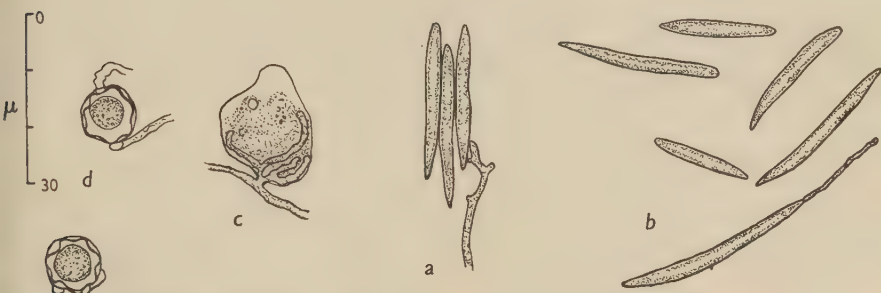


Fig. 1. *Stylopaga rhabdospora* Drechsler. *a*, apex of conidiophore and detached conidia; *b*, conidia; *c*, forking haustorium within an amoeba; *d*, mature zygospores.

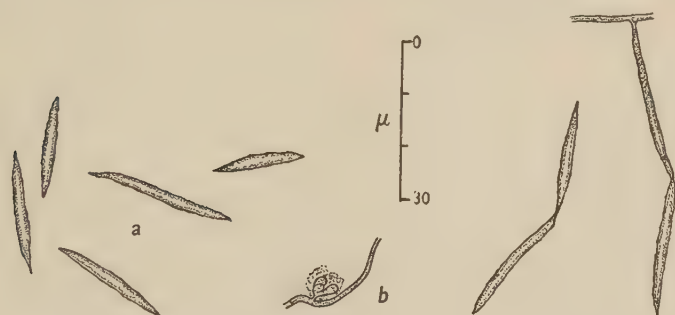


Fig. 2. *Zoopage pachyblasta* Drechsler. *a*, conidia; *b*, haustorium with slightly swollen branches.

Zoopage pachyblasta Drechsler

From a soil sample taken from the bank at the edge of Wisley Pool, Surrey, was isolated a phycomycetous fungus producing chains of filiform conidia. The mycelium was delicate and sparingly branched, and at intervals short lateral branches produced simple or branching chains of conidia. The chains were aerial and most conspicuous under low magnification. Each consisted of many conidia and the distal conidia became detached, falling on the surface of the agar. The conidia were filiform, about $15-32 \times 2 \mu$, distinctly pointed at each end and covered with minute protuberances (Fig. 2*a*). At intervals trapped amoebae were observed, but the small haustoria were difficult to distinguish. Each haustorium was pedicellate and sparingly forked, with rather swollen branches (Fig. 2*b*). No sexual apparatus was observed in this material.

The fungus agrees closely with the description of *Zoopage pachyblasta* given by Drechsler (1947) with which it is identified.

Arthrobotrys cladodes Drechsler var. **macroides** Drechsler

This hyphomycetous fungus which traps eelworms by adhesive networks has been isolated twice. The first sample, of mud and water, was removed at 2½ ft. out from the edge of a pond at Effingham, Surrey; and the second, of soil adjacent to a brackish water area, was taken at Warblington, Hants. After a few weeks on sterile maize-meal agar a Hyphomycete trapping eelworms by networks was observed. Development was similar in the two sets of samples. In young material, small protuberances grew out from the mycelium as short, straight or more commonly curved.

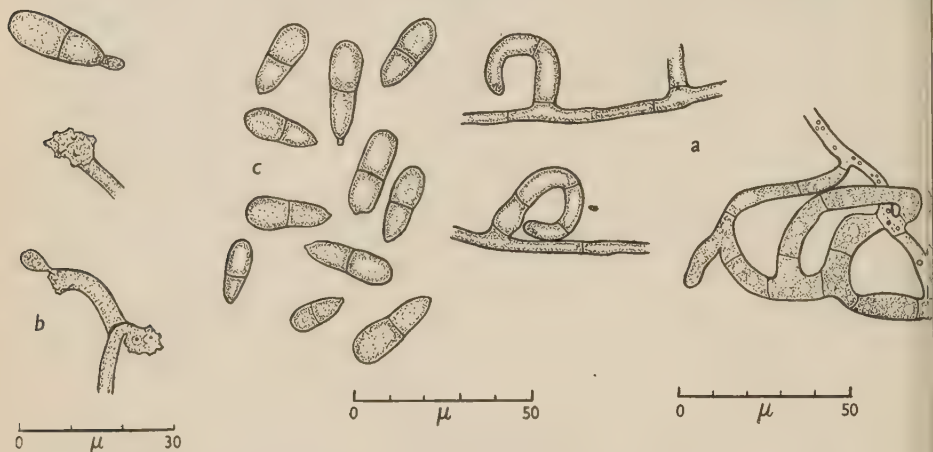


Fig. 3. *Arthrobotrys cladodes* var. *macroides* Drechsler. *a*, short adhesive processes; *b*, branching conidiophore; *c*, mature conidia, two showing basal germ tubes.

processes (Fig. 3*a*). Extensive networks were developed in older material, and many eelworms were trapped. An infection bulb developed within the body of the eelworm, and from it were produced assimilative hyphae which invaded the carcass, and effected digestion and absorption. Conidia were produced on aerial conidiophores. Branching of the conidiophore was of common occurrence (Fig. 3*b*), but no successive nodal formation of conidia was ever observed. Each terminal group contained from five to about twenty bicellular conidia.

Each conidium had a definite basal apiculum (Fig. 3*c*). The conidia ranged from $14 \text{ to } 27 \times 5 \text{ to } 10 \mu$, but few conidia exceeded 9μ in diameter.

The fungus grew well in pure culture on maize-meal agar and rabbit-dung agar and produced an abundance of conidia.

After 6 weeks on maize-meal agar enlarged hyphae were observed. Swollen short cells filled with yellowish globules were formed which probably functioned as storage hyphae.

The fungus agrees well with the description of *Arthrobotrys cladodes* var. *macroides* given by Drechsler (1944), for which the conidial size is stated to be $13\text{--}26 \times 5\text{--}8.2 \mu$. The occurrence of a few conidia measuring 10μ in diameter in the British isolates does not justify the erection of a new species or variety, as in all other respects there is close agreement between the American and British forms.

***Arthrobotrys superba* Corda**

This interesting fungus was isolated from samples of leaf debris, mud and soil taken from Wisley Pool, Surrey. It was found in two sets of samples, one taken 2 ft. from the edge, and the other from 2 ft. out into the pool.

After 3 months, a predacious Hyphomycete trapping eelworms by adhesive networks was observed (Fig. 4*a*). Erect hyphae were produced, each bearing a terminal group of 2-celled conidia, and occasionally more than one group of conidia was produced on a single conidiophore.

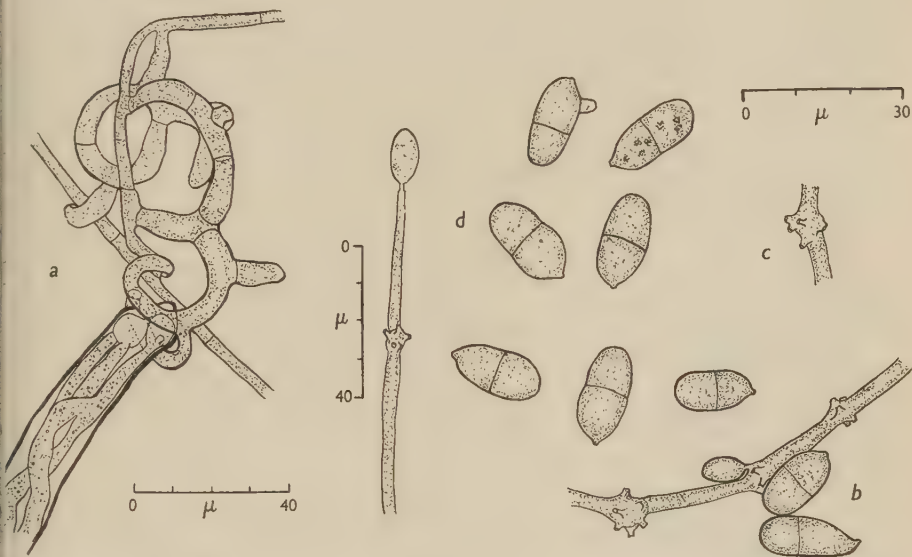


Fig. 4. *Arthrobotrys superba* Corda. *a*, network and captured eelworm; *b*, conidiophore showing swollen nodes with sterigmata; *c*, sterigmata at swollen node; *d*, conidia, one showing germ tube.

The fungus sporulated freely in pure culture on maize-meal agar and on pea-meal agar, and each conidiophore bore successive groups of conidia (Fig. 4*b*). At the point of origin of each conidial group the conidiophore was swollen, and after detachment of the conidia conspicuous sterigmata were observed (Fig. 4*c*). Frequently the conidiophore was branched. Very large numbers of conidia were produced by the fungus under cultural conditions.

A central inoculum of eelworms and three separate inocula of the fungus were placed on sterile pea-meal agar, and after 2 weeks had elapsed, the fungus had produced networks freely all over the surface of the agar. Large numbers of eelworms were trapped, and only one or two living eelworms were seen. The fungus sporulated freely. Clearly under such conditions the fungus is a most efficient destroyer of eelworms.

The size range of conidia in eelworm culture was $12-20 \times 7-10 \mu$, and each conidium was divided into two more or less equal cells (Fig. 4*d*).

The fungus agrees well with the description of *A. superba* Corda given by Drechsler (1937). The size range of conidia as given by this author is $12-23 \times 6.5-9.5 \mu$, and emphasis is laid on the appearance in pure culture of single, irregularly geniculate, nodose axes which may branch.

Dactylaria brochopaga Drechsler

A sample of loam rich in leaf debris was removed from the bank of a water-filled ditch at Watermeads, Mitcham, Surrey, and placed on sterile rabbit-dung agar.

Ten weeks elapsed before fertile hyphae were observed projecting from the inoculum. Each conidiophore bore a small group of slender, septate conidia. A mycelium of septate cells was lying on or just below the surface of the agar, and fallen conidia were fairly abundant. Several conidia had germinated, and on occasion a stalked ring was produced directly (Fig. 5*a*).

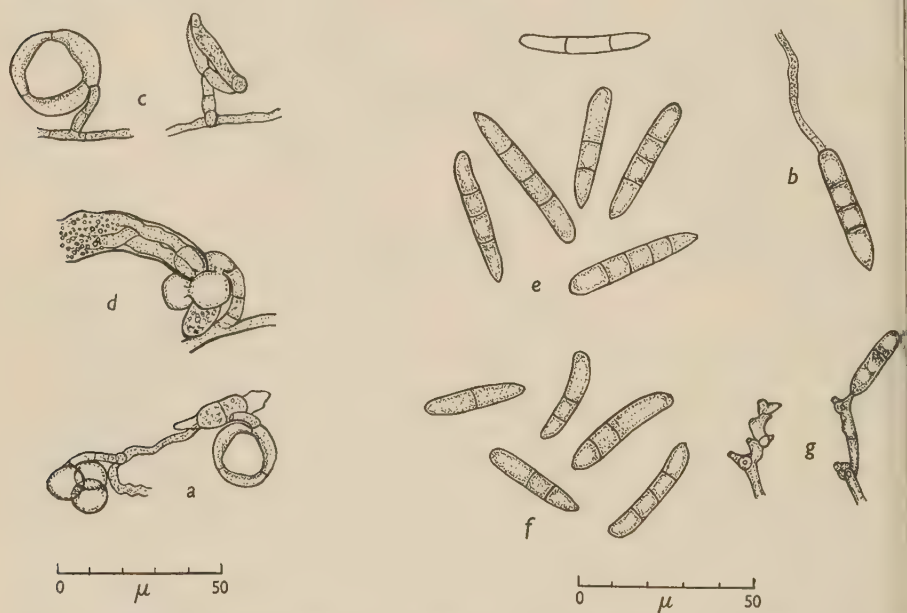


Fig. 5. *Dactylaria brochopaga* Drechsler. *a*, germinated conidium showing stalked ring, and mycelium bearing inflated stalked ring; *b*, conidium showing apical germination; *c*, stalked constricting rings; *d*, eelworm trapped by constricting ring; *e*, conidia; *f*, uniseptate conidium; *g*, apex of conidiophore.

In other cases the germ tube had produced a new mycelium (Fig. 5*b*) which bore stalked rings, each ring consisting of three arcuate cells and the size range was $20-40 \mu$ in external diameter. Each ring was borne on a short stalk of one, two or occasionally three cells (Fig. 5*c*). Here and there the three cells composing the ring had become swollen (Fig. 5*a*), but no eelworm was trapped. In other cases, an eelworm was trapped and constricted by the swollen cells of a ring, an infection tube produced, and assimilative hyphae protruded into the body of the eelworm (Fig. 5*d*). Few eelworms were present in this culture.

Each conidium was septate, and the number of cells ranged from three to five, but four was the usual number. The conidia (Fig. 5e) were elongate and slender with blunt apices and attenuate bases, and commonly the two terminal cells were longer than the central ones. The size range was $29-48 \times 5-8 \mu$.

The fungus grew well in pure culture on maize-meal agar, and produced many conidiophores. Each bore a terminal group of five to ten conidia. The size range of conidia in pure culture was $20-45 \times 4-7 \mu$, and a number of uniseptate conidia were found (Fig. 5f). The apex of a conidiophore from which conidia had fallen was commonly lobed and extended, and successive grouping of conidia was not uncommon (Fig. 5g). A few stalked rings were produced in pure culture.

The arrangement and septation of the conidia is characteristic of the genus *Dactylaria* Saccardo. The shape and size range of conidia agrees closely with that given by Drechsler (1937) for *D. brochopaga* as $26-46 \times 5-9 \mu$.

This species was also identified from a second sample taken at Watermeads, this sample being of mud and water from the ditch itself.

***Acrostalagmus zeosporus* Drechsler**

This endozoic Hyphomycete has been identified from two different situations: from Watermeads, Mitcham, Surrey, and from the bank of the River Thames at Sunbury, Middlesex.

Soil samples were used as inocula on sterile maize-meal agar, and after 1 month dead eelworms were observed in both sets of plates. Dead eelworms were lying on the surface of the agar and others were embedded below the surface. Each eelworm contained septate assimilative hyphae with granular contents, with reproductive branches projecting through the cuticle. In most of the specimens examined the reproductive hyphae were lying horizontally, but on occasion, hyphae protruded into the air. Swollen flask-like phialides were arranged singly, in pairs or in whorls on the reproductive hyphae. The size range of phialides was $5-9 \mu$ in total length and the diameter was about $2.5-3 \mu$. Each phialide was extended distally into a slender neck upon which conidia ($3-4 \times 2 \mu$) were produced successively and cohered to form a terminal group. Each conidium was broadened distally and narrowed towards the base, and resembled a slender maize grain in general shape. On careful examination, eelworms were found which had conidia attached to the surface of the cuticle, but no germ tubes were visible.

The fungus is clearly a member of the endozoic series of Hyphomycetes. *Acrostalagmus zeosporus* Drechsler (1946b) is said to have conidia which resemble maize grains in shape, but the size range is given as $3.5-4.6 \times 1.7-2.1 \mu$ and the size range of phialides as $5-20 \times 2.5-4 \mu$. *A. obovatus* Drechsler (1941) has ellipsoid or obovoid conidia with a size range of $3 \times 2 \mu$. The shape of the conidia in the British forms agrees most closely with that described for *A. zeosporus* with which it is identified.

***Dactylella spermatophaga* Drechsler**

A sample of waterlogged soil containing plant debris was taken on the bank 3 ft. from the edge of a fast-flowing stream near Southwick, Hants, and used as an inoculum on sterile maize-meal agar plates. After a period of 2 weeks a hyphomycetous fungus producing septate conidia was conspicuous around the inoculum. Conidia were produced abundantly, and each was borne singly on a short erect conidiophore (Fig. 6*a*). The septate conidiophores were 30–50 μ in length. Each *Fusarium*-like conidium (28–59 $\times$ 3–5 μ) was septate, slender and commonly tapering at both ends. Frequently, the distal end was less tapering and slightly curved (Fig. 6*b*).

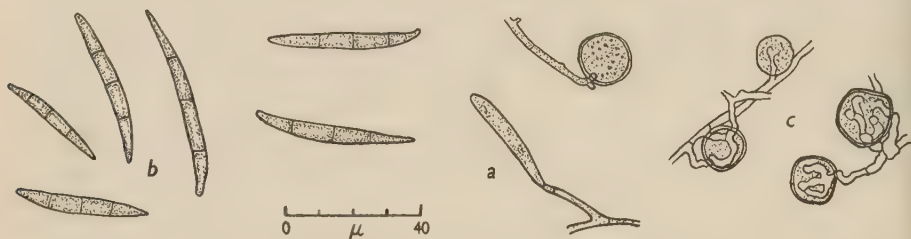


Fig. 6. *Dactylella spermatophaga* Drechsler. *a*, developing conidium; *b*, mature conidia; *c*, haustorial branches within reproductive bodies of *Pythium* sp.

The number of cells was commonly four; rarely two, three or five. In subcultures it was found that the fungus was parasitic on reproductive bodies of a species of *Pythium*. Oogonia, and less commonly sporangia, were invaded by haustorial-like branches (Fig. 6*c*), produced from a hyaline more closely septate region of the hyphomycetous mycelium. In early stages of infection the haustorial branches were difficult to observe, but in later stages were most conspicuous. The protoplasmic contents of the reproductive bodies of the Oomycete were completely absorbed.

The fungus agrees closely with the description of *Dactylella spermatophaga* given by Drechsler (1938). In pure culture experiments using species of *Pythium* Drechsler found that *Dactylella spermatophaga* is definitely a parasitic fungus, attacking oospores of oomycetous fungi.

I should like to express my thanks to Dr B. Barnes for his invaluable advice during the course of this work.

The material here presented formed part of a thesis for the Ph.D. degree in the University of London.

REFERENCES

- CORDEA, A. C. J. (1839). *Pracht-Flora europaeischer Schimmelbildungen*.
 DRECHSLER, C. (1936). New conidial Phycomycetes destructive to terricolous Amoebae. *Mycologia*, **28**, 363–389.
 DRECHSLER, C. (1937). Some Hyphomycetes that prey on free-living terricolous nematodes. *Mycologia*, **29**, 447–552.
 DRECHSLER, C. (1938). Two hyphomycetes parasitic on oospores of root-rotting oomycetes. *Phytopathology*, **28**, 81–103.
 DRECHSLER, C. (1941). Some hyphomycetes parasitic on free-living terricolous nematodes. *Phytopathology*, **36**, 773–802.

- DRECHSLER, C. (1944). Three Hyphomycetes that capture nematodes in adhesive networks. *Mycologia*, **36**, 138-171.
- DRECHSLER, C. (1946*a*). Three new Zoopagaceae subsisting on soil amoebae. *Mycologia*, **38**, 120-143.
- DRECHSLER, C. (1946*b*). A new hyphomycete parasitic on a species of nematode. *Phytopathology*, **36**, 213-217.
- DRECHSLER, C. (1947). Three new species of Zoopage predaceous on terricolous rhizopods. *Mycologia*, **39**, 379-408.
- PEACH, M. (1948). *Nature, Lond.*, **162**, 148-149.
- PEACH, M. (1950). Aquatic predacious fungi. *Trans. Brit. mycol. Soc.* **33**, 148-153.
- PEACH, M. (1952). Aquatic predacious fungi. II. *Trans. Brit. mycol. Soc.* **35**, 19-23.

(Accepted for publication 21 October 1953)

NOTES ON BRITISH UREDINALES

BY MALCOLM WILSON AND D. M. HENDERSON

Royal Botanic Garden, Edinburgh

Hyalopsora adianti-capilli-veneris, *Nyssopsora echinata*, aecidia of *Puccinia arrhenatheri*, *P. confinis*, *P. praecox*, *P. pratensis*, *Uredo oncidii* and *Uromyces aloes* are described in full for the first time in Britain. New host records for Britain are: *Chrysomyxa rhododendrii* on *Picea sitchensis*, *Rhododendron ferrugineum* and *R. roylei*; *Coleosporium tussilaginis* on *Parentucellia viscosa*; *Puccinia arenariae* on *Stellaria nemorum* and *S. uliginosa*; *Puccinia rubigo-vera* on *Agropyron donianum*; *Puccinia veronicae* on *Veronica spicata hybrida*; *Pucciniastrum epilobii* on *Abies grandis* ssp.; *Pucciniastrum pyrolae* on *Ramischia secunda*; *Thekopsora vacciniarum* on *Oxycoccus palustris*; and *Uromyces loti* on *Lotus tenuis*.

These notes include additional details of records of rust fungi which have been made since the publication of Grove's *British Rust Fungi*, 1913, and which appear in the list by Wilson & Bisby (1954). Where species have not been described previously in Britain full descriptions of them are given. The nomenclature of hosts follows Clapham, Tutin & Warburg (1952).

Chrysomyxa rhododendri de Bary Previous to 1940 this species was recorded on *Picea abies* and *Rhododendron hirsutum* (see Grove, 1917; Wilson, 1934). Since then it has also been found on *Picea sitchensis* and a number of rhododendron species. The more important records are: On *Picea sitchensis* near Aberdeen 1952. *Picea abies*, *Rhododendron ponticum* and *R. ferrugineum* in the vicinity were also heavily infected. On *Rhododendron ponticum* in Ayrshire, Kincardineshire, Aberdeenshire and Inverness-shire, on *R. roylei* in Cornwall and on *R. ferrugineum* near Aberdeen, 1951. In Northern Ireland it was collected on *Picea abies* and *Rhododendron ponticum* in 1930 and 1952.

Coleosporium tussilaginis (Pers.) Lév. On *Parentucellia viscosa*, Devon, August 1929; coll. E. J. Cadman (Herb. M. Wilson). Mochrum, Wigtownshire, September 1951; coll. J. Anthony.

These are new host records for this species in Britain. Da Camara *et al.* (1940) have reported it on *P. viscosa* in Portugal. By examining the specimens of *P. viscosa* in the Edinburgh herbarium two more localities have been found: Ansdell near Lytham, Lancashire, August 1908; near Cofton, S. Devon, August 1915.

Hyalopsora adianti-capilli-veneris Sydow On the leaves of *Adiantum capillus-veneris*, Isle of Aran, Galway, 1843. *Uredosori* hypophyllous, producing spots on the upper surface, scattered, small rounded or elliptical, 0.2–0.4 mm. in diameter, often on the nerves, covered by the epidermis, orange-yellow, surrounded by a few paraphyses; spores globose, ellipsoid or angular globose, minutely verrucose, of two kinds: (1) $20\text{--}34 \times 16\text{--}25 \mu$, epispore hyaline $1\text{--}1.5 \mu$ thick, with four equatorial

pores, (2) amphispores, $23.6-31 \times 14.1-21.3 \mu$, average $27.8 \times 19.2 \mu$, epispore $2.6-3.1 \mu$ thick, slightly brownish with 5-6 indistinct pores.

Uredo polypodii on *Adiantum capillus-veneris* was recorded by Plowright (1889, p. 256), and Grove (1913) suggested that this was probably *Hyalopsora adianti-capilli-veneris*. As this rust has been recorded from Austria, Italy, Spain and France it appeared possible that it might be found on British specimens of its host. Accordingly, a search was made in the Edinburgh Herbarium and a few uredosori were found on a specimen of *Adiantum capillus-veneris* collected by Moore from limestone rocks, South Isle of Aran, Galway, in 1843. The uredosori are on leaves bearing mature sporangial sori and so the specimen must have been collected in the latter part of the year. The uredospores in this specimen are usually considerably thicker walled than those described by Sydow (1.5μ) and the wall is slightly brownish; they must be regarded as amphispores and in consequence this species agrees with *Hyalopsora aspidiotus* and *H. polypodii* in possessing two kinds of spores. It appears, however, that intermediate spore forms exist; the uredospore measurement given above are taken from Sydow & Sydow (1915), but in a specimen issued by Jaap, *Fungi selecti exsiccati*, no. 570, the spores are $21.6-31.3$ (28) $\times$ $12.3-20.7$ (16.3) μ and the wall $1.3-2.3 \mu$ in thickness. The amphispore measurements given above are from the Irish specimen. There appears to be no definite peridium, and according to Sydow (*loc. cit.*) its place is taken by a few paraphyses.

Melampsora tremulae Tul. The connexion of the caeomata on *Mercurialis perennis* and the uredo- and teleuto-stages on *Populus tremula* was first demonstrated experimentally by Rostrup in 1883. Plowright (1889) tried to repeat the experiment, but failed to get infection on *Mercurialis* so that the relationship has not been demonstrated experimentally in Britain. In June 1951 plants of *Mercurialis* bearing caeomata were collected in Glen Lyon, Perthshire. Spores from this collection were used to inoculate *P. tremula*, and within 1 month uredo- and teleuto-spore stages agreeing with those described for *Melampsora tremulae* had developed.

Nyssopsora echinata (Lév.) Arth. On *Meum athamanticum*, Glen Lyon, Perthshire, July 1939; coll. M. Y. Orr. Lawers, Perthshire, July 1948 and 1951 (D. M. H.). Finegand, Glen Shee, Perthshire, July 1952 (D. M. H. 127).

The sori on the leaf segments are small and rather inconspicuous and those on the petioles often occur near the base so that plants have to be examined very closely to find the fungus. Spermogonia have never been described for this species. A careful search for them on the 1952 collection did not reveal any.

Teleutosori on the petioles and leaf segments 1.5×1.2 mm., producing little distortion, slightly pulverulent, covered by the epidermis which ruptures early and remains as a conspicuous fringe about the sorus; *teleutospores*, black in mass, each composed of three cells of more or less equal size triangularly arranged; spores, flattened laterally $17.5-22.5$ (20.0) μ thick, $25-30$ (28.5) $\times$ $23-27$ (25.4) μ . Pedicel, inserted on the single basal cell, hyaline, rather fragile, short. Spore cell walls, dark

chestnut brown, covered, especially on the somewhat flattened, peripheral wall, with conspicuous curved spines 6.6–12 (8.6) μ long, tapering outwards, frequently minutely dichotomous at the apex. Pores usually two in each cell, lateral near the inner angles.

The species was first recorded from Britain by Wilson (1940) without details. It has been recorded on *Meum athamanticum* in Europe from Germany, Austria, Hungary, Switzerland and France (Sydow & Sydow, 1915). Hagen (1941) has recorded it recently on herbarium specimens from two localities in Norway.

Puccinia albulensis Magn. The collection by Berkeley mentioned by Grove (1913, p. 170) is undoubtedly this species. Specimens from the same locality—Ben Alder, Inverness-shire—were collected in 1940. In 1953 pulvinate teleutosori on systematically infected sterile shoots of *Veronica alpina* were found in Caenlochan Glen, Angus. (D. M. H. 751). Jørstad (1931) considers that *Puccinia albulensis* is confined to *Veronica alpina* and that no other rust occurs on that host.

Puccinia arenariae (Schum.) Wint. The following are additional host records for Britain:

On *Stellaria uliginosa*. Gairloch, W. Ross. August 1934 (M. W.).

On *Stellaria nemorum*. Roslin, Midlothian, 1935 (M. W.), Glasbeath, Cardigan, 1924 (J. Salter, specimen in Herb. Nat. Mus. Wales).

Puccinia arrhenatheri (Kleb.) Erikss. The first record of the aecidial stage in Britain is by R. W. G. Dennis who found it on *Berberis vulgaris* in Northumberland in 1944.

Spermogonia very numerous, minute, covering a great part or the whole of the leaf uniformly. *Aecidia* hypophyllous, sometimes even on the flowers, deforming the affected branches, generally distributed densely and evenly over the whole leaf, between cylindrical and cup-shaped, with a torn whitish revolute margin; spores subglobose or ellipsoid, verruculose, yellowish, 19–32 $\times$ 16–24 μ .

This species produces on the barberry its characteristic witches' brooms, composed of many upright branches. An account of the structure and distribution of the mycelium in the stems and buds of the barberry has been given by Magnus (1898).

The aecidium is distinguished from that of *Puccinia graminis* by the larger, distinctly warted spores, the more strongly developed prismatic structure of the inner wall of the peridium (the outer wall is very thick) and the less distinct spore chains.

Puccinia asteris Duby Grove assigned the British rust on *Aster tripolium* to *Puccinia tripolii*. He stated that the latter differs from the American specimens of *P. asteris* in the absence of spots, in the occurrence of the sori all over the leaf surface and in minor differences in the teleutospores.

The stomata of *Aster tripolium* are generally distributed over the leaf surface. As the sori arise beneath the stomatal openings they have a similar

distribution. The absence of spots on *A. tripolium* is merely a host reaction. The differences in teleutospore structure mentioned by Grove do not appear to be taxonomically significant.

Puccinia clandestina Carm. The specimen in the Kew Herbarium collected by Carmichael from Appin and described by Berkeley (*English Flora*, 1836, p. 365) as on *Succisa pratensis* is on *Solidago virgaurea*. It is undoubtedly *Puccinia virgaureae*. The specimen of '*P. clandestina*' recorded by Rilstone (1938) from Cornwall consists of a leaf of *Solidago* bearing a few very small sori. It should also be referred to *Puccinia virgaureae*.

Puccinia confinis Sydow Spermogonia and aecidia on *Solidago virgaurea*, Ben Vaar, Argyll, about 2800 ft., September 1942 (M. W.). Prof. Heslop Harrison informs us that he discovered aecidia on *S. virgaurea* on the island of Rassay in 1936.

Spermogonia, epiphyllous on pale yellow spots. *Aecidia*, hypophyllous in small groups on yellow spots on the upper leaves. Peridium white with revolute, lacinate margin. Aecidiospores $16.5-20.5 \times 15-18 \mu$.

The uredo- and teleuto stages have not been found in Britain. They were described by Sydow & Sydow (1920) from Latvia.

Aecidia were found in Norway in 1933 by Hagen (1941) and were described under *Puccinia dioicae* Magn. They were again found in the same locality in 1939 by Jørstad (1943) who also found the teleuto-stage on *Trichophorum caespitosum* with a few uredospores in the teleutosori. Jørstad carried out successful inoculations with teleutospores on *Solidago virgaurea* and produced spermogonia and aecidia.

Puccinia graminis Pers. Teleutospores were collected on *Deschampsia caespitosa* from Pickering, Yorks, by W. G. Bramley, December 1952. They are uncommon on this host.

Puccinia libanotidis Lindr. An early collection on *Seseli libanotis* is in the Edinburgh Herbarium made by S. H. Bickham from Cherry Hinton, Cambs, 1901.

Puccinia liliacearum Duby On *Ornithogalum umbellatum*, Whitekirk, E. Lothian, 1935. Roxburgh, 1941 (M. W.). Blairgowrie, Perthshire, 1950 (D. M. H.). Previous records refer only to England.

Puccinia obscura Schroet. On *Luzula forsteri*, East Grinstead, Sussex, May 1953, P. H. Davis, 16,829. Once recorded on this host in Cornwall (Rilstone, 1938).

Puccinia praecox Bubák This species was found by W. G. Bramley on *Crepis biennis* at Bolton Percy, Yorks, August 1941. Only uredospores were present. Teleutospores were collected in 1942 but spermogonia and aecidia have not been found. As the latter probably occur in Britain a description of them is included. On the continent a second aecidium, that of *Puccinia silvatica*, has been found on *Crepis biennis*. The two aecidia may be distinguished by the structure of the peridium; the peridial cells in *Puccinia*

praecox are not in regular rows while those of *P. silvatica* are broadly ellipsoidal, arranged in very regular rows.

Spermogonia, amphigenous, scattered or in groups between the aecidia, honey-coloured. *Aecidia*, amphigenous, on yellowish or reddish, irregular on rounded spots, rarely on the nerves, small, cupulate with reflexed denticulate peridium; peridial cells ellipsoid or elongate-deltoid, not in regular rows; aecidiospores polygonal-globose, ovoid or oblong, finely verruculose, orange, $17.5-31 \times 17.5-22 \mu$. *Uredosori*, amphigenous or on the stem, on yellowish spots, minute, pulverulent, brown; uredospores globose, ellipsoid or ovoid, aculeate, brown, $22-33 \times 20-29 \mu$, with two or rarely three pores. *Teleutosori*, amphigenous on yellowish spots, minute, solitary or often confluent, soon naked, pulverulent, blackish brown; teleutospores widely ellipsoid or ovoid, rounded at both ends; apex unthickened, scarcely or not constricted, finely verruculose, deep brown, $30-46 \times 24-31 \mu$; epispore thin with pores depressed about half or more down the cells; pedicel short, hyaline, deciduous.

Puccinia pratensis Blytt Uredospores Aug.-Sept. and teleutospores Sept.-Oct. on *Helictotrichum pratense* (= *Avena pratensis*). A few mesospores were present. Yorkshire, W. G. Bramley, autumn 1952.

In Norway, it extends northwards beyond the Arctic Circle; there, as in Yorkshire, it is found on calcareous soil.

Uredosori and *teleutosori* hypophyllous, scattered, minute or medium in size, ellipsoid, oblong or oblong-linear, surrounded by the ruptured epidermis, pulverulent, ferruginous; *uredospores* globose or obovate, coarsely echinulate, yellowish brown, $27-31 \times 25-27 \mu$; epispore clear chestnut-brown, $2-3 \mu$ thick with 5-7 pores; *teleutospores* broadly ellipsoid or ovate, rounded at both ends, scarcely constricted, densely verrucose, chestnut-brown, $36-47 \times 25-28 \mu$; epispore $3-7 \mu$ thick, pore of upper cell more or less apical, of lower depressed to middle or more; pedicel short, thick, fragile.

Jørstad (1950, p. 47) has stated that this species differs from all other Northern European grass rusts by its verrucose teleutospores and deciduous pedicles, but three species have been described which closely resemble *Puccinia pratensis* and, as they grow on different species of *Helictotrichum*, may possibly be regarded as biological races; these are *Puccinia versicaloris* Semadini from Switzerland and North Italy, *P. avenastri* Guyot *et al.* and *P. bromoides* Guyot *et al.* from France (Guyot *et al.* 1951, p. 141).

The warts on the teleutospores of *P. pratensis* are relatively large and rounded with small inter-spaces; approaching the base of the lower cell they become slightly elongated towards the pedicel.

Puccinia pygmaea Erikss. Collected on *Calamagrostis epigeos*, Dunkeld, Perthshire 1953 (D. M. H. 891). Uredospores only. Rust on *Calamagrostis* has not been recorded previously in Scotland. Wilson & Bisby (1954) record it from Yorks, Worcs and Bucks.

Puccinia rubigo-vera Wint. Uredo- and teleutosori on *Agropyron donianum* Meall nan Tarmachan, Lawers, Perthshire, August 1953 (D. M. H. 909).

This collection is provisionally assigned to the collective species *Puccinia rubigo-vera*. The teleutosori were abundant, suggesting that the fungus is host alternating. It may belong to the *P. borealis* group alternating with *Thalictrum* spp. *T. alpinum* was growing in the vicinity of infected plants but no infection was noted.

Puccinia veronicae Schroet. On *Veronica spicata* ssp. *hybrida*. Infected plants collected from Humphrey Head, Lancs, were grown by W. G. Bramley in Yorkshire. This material fits *Puccinia veronicae* as defined by Gäumann (1941).

Puccinia veronicarum DC. Grove listed *Veronica officinalis* as a host for this species. No specimens can be traced. The host was probably misidentified. The other collection mentioned by Grove as, 'on *V. alpina*', is *Puccinia albulensis*.

Pucciniastrum epilobii Otth. On *Abies grandis*, Spottiswoode, Lauder, 1951 (M. W.). Inoculations with teleutospores from *Chamaenerion angustifolium* were made in 1950 and spermogonia and aecidia produced on *Abies grandis*. In the following year aecidia were found in nature in a plantation of young trees of *A. grandis* near Lauder.

Pucciniastrum pyrolae Diet. ex Arth. On *Ramischia secunda*, Caenlochan Glen, Angus, 2500 ft. July 1953 (D. M. H. 750). Only uredospores were present. A new host record for Britain.

Thekopsora vacciniorum Karst. On *Oxycoccus palustris*, Ardrishaig, Argyll, June 1953 (D. M. H. 731). Previously recorded from Northumberland on this host. Only uredosori present.

Uredo oncidii P. Henn. On *Oncidium cavendishianum*, Royal Botanic Garden, Edinburgh, 1938 (M. W.). The uredospores in this collection are considerably larger ($22-30 \times 15-22 \mu$) than those of *Uredo behnickiana* P. Henn ($16-18 \times 20 \mu$). Previously recorded as possibly occurring in Britain by Wilson & Bisby (1954).

Uromyces aloes (Cooke) Magn. On *Aloes glauca* Mill. and three other unnamed species of *Aloes* in greenhouses, Royal Botanic Garden, Edinburgh, September 1938 (M. W.).

Teleutosori, amphigenous, developing in concentric rings, forming large discoloured spots 2-3 cm. across on the leaves; sori 1-2 mm. in diameter surrounded by the conspicuous torn epidermis; teleutospores, in mass vandyke brown, $29-34 (31) \times 26-30 (28) \mu$; spore wall, smooth, uniformly thickened, 3-4 μ thick; pedicel, hyaline, usually persisting as a short peg.

This species has been recorded on fifteen species of *Aloes* and three species of *Haworthia* in South and Central Africa (Doidge, 1950) and on two species of *Aloes* in India (Thirumalachar, 1947). According to the latter author's recent investigation of the cytology of this species, it is a microcyclic species with teleutospores and spermogonia only.

Uromyces armeriae Lév. On *Armeria maritima* var. *alpina* Lawrence, *A. maritima* var. *elongata* Massart and *A. plantaginea* Willd. var. *plantaginea*, Royal Botanic Garden, Edinburgh.

This rust has been recorded on *A. maritima*, *A. grandiflora* and *A. leucocephala* in Scotland (Wilson, 1934). The herbarium specimens and plants in the Garden have been examined. However, none of the specimens in Greville's collection belong to *A. leucocephala* but are most probably *A. plantaginea* Willd. var. *plantaginea*, and plants in the garden under *A. leucocephala* are certainly this latter species. The specimen of *A. grandiflora* in Greville's herbarium match that species quite well, and should be called *A. pseudo-armeria* Lawrence, according to Lawrence's recent synopsis of the species of *Armeria* (Lawrence, 1940).

A number of collections have been examined and the following spore forms found: *A. maritima* var. *alpina* I, II and III; *A. maritima* var. *elongata* I, II and III; *A. plantaginea* var. *plantaginea* I and II; *A. maritima* var. *maritima* I, II and III.

Savile & Connors (1951) have recently divided *Uromyces armeriae* in North America into a number of geographically distinct subspecies. The disjunctions between the subspecies follow closely the overlapping of the areas of distribution of the various host species. All the Scottish material is referable to their subspecies *U. armeriae* ssp. *armeriae*.

As Savile & Connors (1951) remark, the life cycle of this species is incompletely understood. Spermogonia were rare in their collections of ssp. *hudsonius* and absent in ssp. *pacificus*.

All the Scottish material was carefully examined but spermogonia were found on only one leaf of a plant which has long been labelled *Armeria dianthifolia* in the Royal Botanic Garden, Edinburgh. This name cannot be traced, and according to Lawrence's key the plant is *A. maritima* var. *alpina*. The spermogonia were abundant on this leaf, epiphyllous, honey-coloured, c. $40 \times 40 \mu$. Spermatia approximately $1 \times 0.5 \mu$.

***Uromyces loti* Blytt** On *Lotus tenuis*, Eastbourne, July 1953; P. H. Davis, 16,717. Uredospores only. This is a new host for this species in Britain, it has been recorded previously on *L. angustissimus*, *L. corniculatus* and possibly *L. hispidus*.

REFERENCES

- CAMARA, S. DA *et al.* (1940). *Uredinales aliquot Lusitaniae*. *Agron. lusit.* **2**, 113-167.
 CLAPHAM, A. R., TUTIN, T. G. & WARBURG, E. F. (1952). *Flora of the British Isles*. Cambridge University Press.
 DOIDGE, E. M. (1950). The South African fungi and lichens. *Bothalia*, **5**, 1-1094.
 GÄUMANN, E. (1941). Ueber die *Puccinia* auf *Veronica spicata*. *Ann. mycol., Berl.*, **39**, 39.
 GROVE, W. B. (1913). *British Rust Fungi*. Cambridge University Press.
 GROVE, W. B. (1917). Mycological notes. *J. Bot., Lond.*, **55**, 134-136.
 GUYOT, A. L. *et al.* (1951). Sur deux espèces nouvelles d'Uredinées. *Uredineana*, **3**, 141-150.
 HAGEN, A. (1941). Notes on Norwegian Uredineae. *Nytt Mag. Naturv.* **82**, 123-140.
 JØRSTAD, I. (1931). Notes on Uredineae. *Nytt Mag. Naturv.* **70**, 325-340.
 JØRSTAD, I. (1943). The aecidial stage of *Puccinia confinis* Sydow. *Nytt Mag. Naturv.* **83**, 100-103.
 JØRSTAD, I. (1950). The Graminicolous Rust Fungi of Norway. *Skr. norske VidenskAkad.* no. 3.
 LAWRENCE, G. H. M. (1940). *Armerias*, native and cultivated. *Gentes Herb., Ithaca*, **4**, 391-418.
 MAGNUS, P. (1898). On *Aecidium graveolens*. *Ann. Bot., Lond.*, **12**, 155-163.

- PLOWRIGHT, C. B. (1889). *British Uredineae and Ustilagineae*. London.
- RILSTONE, F. (1938). Cornish Microfungi. *J. Bot., Lond.*, **76**, 353-361.
- SAVILE, D. B. O. & CONNERS, I. L. (1951). The rusts of *Armeria* and *Limonium* in North America. *Mycologia*, **43**, 186-195.
- SYDOW, P. & SYDOW, H. (1904). *Monographia Uredinearum*, I. *Lipsiae*, 972 pp.
- SYDOW, P. & SYDOW, H. (1915). *Monographia Uredinearum*, III. *Lipsiae*, 726 pp.
- SYDOW, H. & SYDOW, P. (1920). *Novae fungorum species*, XVI. *Ann. mycol., Berl.*, **18**, 154-160.
- THIRUMALACHAR, M. J. (1947). A cytological study of *Uromyces aloes*. *Bot. Gaz.* **108**, 245-254.
- WILSON, M. (1934). The distribution of the Uredineae in Scotland. *Trans. Edinb. bot. Soc.* **31**, 345-449.
- WILSON, M. (1940). *Trans. Edinb. bot. Soc.* **33**, iv.
- WILSON, M. & BISBY, G. R. (1954). List of British Rust Fungi. *Trans. Brit. mycol. Soc.* **37**, 61-68.

(Accepted for publication 26 October 1953)

FURTHER DESCRIPTIONS OF LUMINOUS AGARICS

By E. J. H. CORNER

Botany School, University of Cambridge

(With Plate 9 and 12 Text-figures)

The following luminous species, collected by Y. Haneda in Asia, are described: *Dictyopanus gloeocystidiatus* sp.nov. (Japan), *Mycena chlorophos* (Berk. & Curt.) Sacc., *M. illuminans* P. Henn., *M. lux-coeli* sp.nov. (Japan), *M. noctilucens* sp.nov. (Micronesia), *M. pruinoso-viscida* sp.nov. (Malaya) and var. *rabaulensis* var.nov. (New Britain), *M. sublucens* sp.nov. (Amboina), *M. (Poromycena) manipularis* (Berk.) Métrod and var. *microporus* var.nov. (Micronesia). Microscopic details are given for *Dictyopanus pusillus* (Lév.) Singer and var. *sublamellatus* var.nov., both from South America. Types are preserved in the Cambridge University Herbarium.

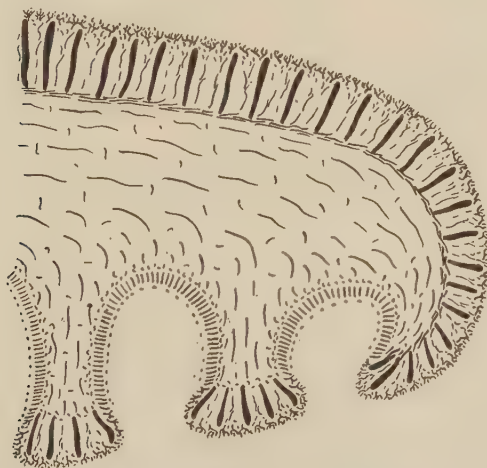
The taxonomist has inherited a wearisome tangle of synonyms among tropical fungi. Strictly he should clear this up before describing miscellaneous new species. But other mycologists need names to record their researches and cannot wait for the counsel of perfection. For this reason, I describe five new species of agaric with luminous fruit-bodies or mycelia, in order to assist Dr Y. Haneda in his enthusiasm for bioluminescence. Most of these fungi were known to me in Malaya before Dr Haneda brightened our war-years, and I can give, therefore, fuller accounts than usual, which will help subsequent monographers. Dr Haneda's biophysical researches, published in Japanese, are little accessible, but I understand that, on the completion of the taxonomic side, he will re-edit his work. I am indebted to him for excellent material, preserved in alcohol-formalin, and for many photographs, and I cannot refrain from insisting on the need of this alcohol-formalin material in studying 'exotic agarics' without access to living material. Unfortunately, the test for amyloid spores does not always work in such material, which is supplementary to the dried specimen, as both are to the living.

DICTYOPANUS PAT.

The species are very similar macroscopically and it is probable that all are luminous. Microscopically there are differences in spore-size and in the form of the cystidia and hyphal endings in the granular encrustation over the surface. For this reason I am compelled to distinguish a Japanese collection, under the name *D. gloeocystidiatus*, from *D. pusillus*, and to query previous identifications of *D. pusillus* from the Eastern tropics. Thus, *D. pusillus* is common in tropical America, but I never saw it in Malaya. Fortunately the encrustation concealing the hyphal ends is readily soluble in dilute potash, and the details can be made out from dried material.



Text-fig. 1. *Dictyophanus gloeocystidiatus*, the surface of the pileus (much simplified) and basidia. $\times 1000$.



Text-fig. 2. *D. gloeocystidiatus*, a diagram of the structure of the pileus to show the occurrence of the gloeocystidia.

Dictyopanus gloeocystidiatus sp.nov. (Text-figs. 1, 2)

Pileus -6 mm. latus, convexus dein applanatus, albus, cretaceo-pruinosis: stipes 0.5-1.3 mm. longus et crassus, pruinosis: tubuli -0.5 mm. longi, poris 70-120 μ latis, ad marginem subelongatus, dissepimentis 70-140 μ crassis. *Sporae* 3.2-4 $\times$ 2-2.3 μ , subcylindricae, obtusae, vix amyloideae; basidia 15-19 $\times$ 3.5-4 μ , clavata, sed juvenilia acerosa. *Gloeocystidia* -60 $\times$ 3.5-4.5 μ , ut cheilocystidia, pileocystidia et caulocystidia sub pruina superficiali, subcylindrica, obtusa vel ad apicem lobis angustis 1-2 praedita, saepe apicem versus 1-2 septata, tenuiter tunicata, pleurocystidiis nullis. *Dichophyses* in pruina superficiali e stiptibus 1.5-2.5 μ latis repetiter subdichotoma, apicibus 0.5 μ latis, dense intricata, ramis diversiformibus, valde incrustata. *Hyphae* nodoso-septatae, crasse, tunicatae, plus minus inflatae -17 μ latae, tunicis -5 μ crassis, extus saepe rugulosis, plerumque longitudinales sed hyphis angustis irregulariter sublobatis intertextae, haud gelatinosae.

Ad lignum: Japan, Ins. Hachijo, leg. Y. Haneda, Jun. 1951.

This species differs from *D. pusillus* in the gloeocystidia and distinct dichophyses. The pores are luminous, as Kobayasi (1937) has described for *D. pusillus*, though his material may prove to be *D. gloeocystidiatus*.

Dictyopanus pusillus (Lév.) Singer (Text-fig. 3)

R. Singer, *Lloydia*, **8**, 224, 1945; *Lilloa*, **23**, 179, 1950; R. W. G. Dennis, *Kew Bull.* p. 326, 1952.

Sporae 3.5-3.7 $\times$ 2.3-2.8 μ : basidia 13-15 $\times$ 4.5-5 μ , sterigmata 4. *Cheilocystidia* -40 $\times$ 4-6.5 μ , subclavate, the walls thin or slightly thickened, rather densely spiculate over the distal half with short, simple or sparingly



Text-fig. 3. *D. pusillus*, pileocystidia and spores ($\times$ 1000): specimen from Brazil (Manaus, 24 Oct. 1948).

branched processes 1-6 $\times$ 1-1.5 μ , sometimes shortly lobed but not digitate, densely incrustated: pleurocystidia none. *Hyphae* 2-5.5 μ wide, some slowly inflating to 11 μ in the pileus and stem, clamped, monomitic, the walls 1-4 μ thick, becoming uneven and kinked, densely interwoven, with the wide and narrow hyphae intermingled, longitudinal in the stem: more or less encrusted with granular matter: 2-4 μ wide in the dissepiments, not gelatinous. *Caulocystidia* as the cheilocystidia, as a loose palisade, densely encrusted. *Pileocystidia* as the cheilocystidia but mostly ill-formed, often decumbent, or merely as hyphal ends with a few short lobes or processes.

var. **sublamellatus** var. nov. (Text-figs. 4, 5)

Poris marginem pilei versus radiatim elongatis v. sublamellatis: caulocystidiis, et pileocystidiis, vix distinctis, ut apicibus hypharum plus minus breviter lobatis: ad lignum, Venezuela, pr. Caracas, c. 1800 m. alt., 11 Oct. 1947, Corner 847 *typus*, 848.

Pileus -2 cm. radius, reniform to flabelliform, minutely cottony-pruinose, white to pale drab fawn, isabelline, or brownish, drying furfuraceous and cracked: stem $-7 \times 1.5-2$ mm., as the pileus: *tubes* -0.5 mm. long, the pores $100-250 \mu$ wide, regular and angular near the stem, radially elongate in the middle part of the fully developed pileus, lamellate-poroid toward the margin, the dissepiments $80-180 \mu$ thick, white, then concolorous, sometimes the pores developed in cyphelliform groups round the stem-apex. *Spores* $3.5-4 \times 2.5-2.8 \mu$, as in *D. pusillus*: basidia $18-25 \times 5 \mu$: cheilocystidia as in *D. pusillus*.



Text-fig. 4. *Dictyopanus pusillus* var. *sublamellatus*, pileocystidia and spores, $\times 1000$; radial section of the fruit-body. $\times 10$.

Surface of the stem and pileus with a slight crust, $40-80 \mu$ thick, composed of $2-3.5 \mu$, oblique or perpendicular, thin-walled hyphal ends, many shortly lobed with processes $-8 \times 1.5 \mu$, but not spiculiferous, some even subclavate, but not as regular cystidia.

I have given these details of the microscopic characters of *D. pusillus* from collections which I made in Brazil, in order that they may be compared with those of *D. gloeocystidiatus* and of *D. luminescens* (Corner, 1950). Var. *sublamellatus* shows that *Dictyopanus* is hardly to be separated from *Panellus*.

MYCENA S. F. GRAY

There are numerous tropical species of the section *Basipedes*, some of which are luminous. The European *M. stylobates* has a luminous mycelium (Wassink, 1948), but *M. chlorophos* and *M. illuminans* have luminous fruit-

bodies. There is some confusion regarding these two species, so that I describe them in detail from my Malayan collections. In Brazil I found commonly a species that is very close to *M. chlorophos*, if not merely a variety, but I failed to note if it were luminous. A common species in Malaya, also close to *M. chlorophos*, but for which I have not found a name, is not luminous. The discoid base of the stem and the gelatinous sub-epidermal layer of the pileus well characterize the section, but as usual



Text-fig. 5. *D. pusillus* var. *sublamellatus*. $\times 10$.

there is much specific variation in the cystidia, both on the gill-edge and on the pileus. Unfortunately the pileocystidia are often lost on expansion of the pileus and swelling of the jelly, so that it is necessary to examine unexpanded fruit-bodies.

Other luminous species of the genus are scattered in various sections. I would draw attention to Jossierand's recent findings with regard to *M. rorida* (1953). *M. pruinoso-viscida*, here described, is closely related and seems to pose the same problem.

Section *Basipedes****Mycena chlorophos*** (Berk. & Curt.) Sacc. (Text-fig. 6)

Saccardo, *Syll. Fung.* **5**, 301, 1887; Kobayasi, Y., *Bull. biogeogr. Soc. Japan* **7**, 6, 1937; Ito, S. & Imai, S., *Trans. Sapporo nat. Hist. Soc.* **16**, 16, 1939.

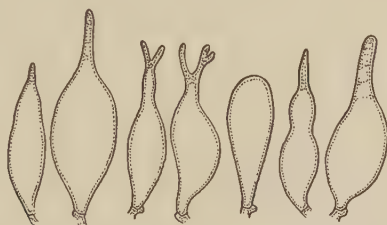
Basinym: *Agaricus* (*Mycena*) *chlorophos* Berk. & Curt., *Proc. Amer. Acad. Arts Sci.* **4**, 113, 1860.

Syn.: *Agaricus* (*Mycena*) *cyanophos* Berk. & Curt., *Proc. Amer. Acad. Arts Sci.* **4**, 113, 1860; Kobayasi, Y., *Bull. biogeogr. Soc. Japan*, **7**, 4, 1937, f. 1., pl. I, f. 3., pl. III; *Bull. nat. Sci. Mus. Tokyo*, **26**, 3-9, 1949, f. 1-2; Haneda, Y., *Kwagaku-nanyo*, **4**, 226, 1942; *Kwagaku-nanyo*, **1**, 1939, f. 3 (as *M. phosphora* Kawamura nom. nud.).

M. cyanophos Berk. & Curt. f. *carolinensis* Y. Kobayasi, *Bot. Mag. Tokyo*, **53**, 161, 1939.

? *M. discobasis* Métrod, *Prodr. Fl. Mycol. de Madagascar* **III**, *Les Mycènes*, 22, 1949, f. 1.

(*M. illuminans* sensu Petch, *Ann. R. bot. Gard. Peradeniya*, **9**, 313, 1925, *haud vera*.)



Text-fig. 6. *Mycena chlorophos*, cheilocystidia. $\times 500$.

Pileus -17 mm. wide, -30 mm. (Ito and Imai), convex then *plano-umbilicate*, *sulcato-rimose* to the disk, *pale fuscous* or *mouse-grey* and *subpruinose*, *paler* or *brownish* then *whitish* on expansion and *subviscid*: margin *crenulato-denticulate*. *Stem* 6-30 $\times$ 0.3-1 mm., tense, hollow, *translucent*, *minutely pubescent*, *white*, the *strigose* or *finely villous* base *discoïd* or *subbulbous*, 1-1.5 mm. wide (-2.5 mm., Ito and Imai). *Gills* free or adnexed to a slight collar, thin, *scarcely crowded* 17-32 primaries, 1-3 (-4) ranks, 0.3-1 mm. wide, *white* then *greyish*, the edge *micaceous*. *Flesh* very thin: *smell ammoniacal*, nitrous, rather strong. *Spores* 7-8.5 (-10) $\times$ 5-6 μ , white, smooth, ellipsoid, with cloudy contents, slightly greyish amyloid. *Basidia* 17-23 $\times$ 7.5-10 μ : sterigmata 4, 3 μ long: *paraphyses* 5-8 μ wide, shorter than the basidia, more abundant and forming a toughly subgelatinous layer. *Cheilocystidia* -60 $\times$ 7-21 μ , as a sterile edge, hyaline, conical or ventricose, obtuse, acute or more usually with a short appendage -15 $\times$ 2-3 μ , occasionally branched, thin or slightly thick-walled: *pleurocystidia* none. *Pileocystidia* 25-60 $\times$ 13-25 μ , clavate, more or less thick-walled, *echinulate* on the exposed surface with short simple processes -3 μ long, more or less cemented together and forming a continuous layer over the young pileus but disrupting on expansion: without other forms of pileocystidia. *Caulo-*

cystidia $-300 \times 10-25 \mu$, conical or lanceolate, subacute, hyaline, smooth, with thin or slightly thickened walls, shorter in the upper part of the stem and passing into the ventricose-appendaged form of the cheilocystidia. *Hyphae* of the stem -35μ wide, *secondarily septate* (without clamps) with cells $40-250 \mu$ long, with narrow uninflated and clamped hyphae intermingled: in the pileus and gills inflated with clamps, the cells $40-90 \times 12-50 \mu$, pseudoparenchymatous, those below the gelatinous layer with pale umber sap: gelatinous layer of the pileus $50-100 \mu$ thick, composed of narrow hyphae ending at the surface in the palisade of pileocystidia: subhymenial hyphae narrow, $1.5-2.5 \mu$ wide, subgelatinous.

Pileus and gills brightly bluish green luminescent, the mycelium and stem scarcely luminous (Haneda, Kobayasi).

On dead twigs, bark and wood, solitary, in the forest: Malaya (common), Ceylon, Japan (Hachijo Island), Bonin Island, Micronesia (Palau, Ponape), Rabaul.

According to my observations in 1934, the fruit-bodies take about 5-6 days to reach the state of a hemispherical primordium, $1.5-3 \text{ mm.}$ wide, consisting of the pileus and stem fully formed, though unexpanded, on the discoid base. The stem begins to lengthen soon after sunrise and is fully grown about an hour after noon. The pileus is not fully expanded, and plane, until sunset. Sporing then occurs overnight and the fruit-body collapses between 10 a.m. and noon on the following (6th or 7th) day. This is the nocturnal habit of the delicate, paraphysate fruit-body to which several lines of Agaricaceae have converged.

I have followed Ito and Imai in their reduction of *M. cyanophos*, for their reduction was earlier than that of Kobayasi who reduced *M. chlorophos* to *M. cyanophos*. The nitrous smell of the fresh fruit-bodies is highly characteristic in my experience, though not recorded by others, and Ito and Imai say 'odour and taste none'. Nevertheless, the Malayan fungus, as well as that collected by Haneda in Micronesia and Rabaul, is certainly the same as *M. cyanophos* of Kobayasi and in no other way distinct from that of Ito and Imai.

In Brazil I collected at Manaus and Rio de Janeiro on numerous occasions a fungus that differs from *M. chlorophos* of Asia merely in the following details:

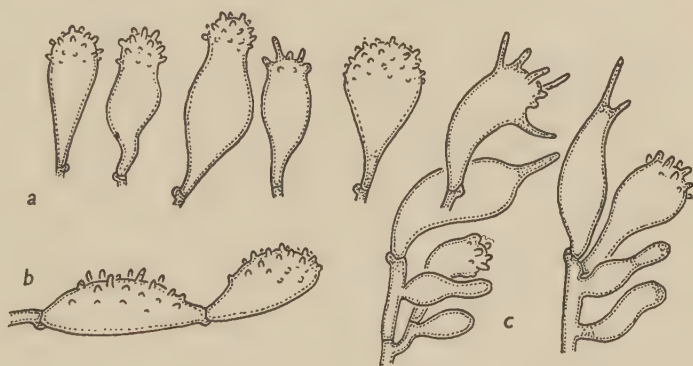
- (1) Slightly larger fruit-body; pileus -35 mm. wide, stem $1-3.5 \text{ mm.}$ wide below, the base more or less discoid $2-4 \text{ mm.}$ wide.
- (2) More numerous, more crowded and much wider gills, 30-40 primaries, $1-5 \text{ mm.}$ wide, often pinkish with age.
- (3) Cheilocystidia without appendage.
- (4) Echinulate cells on the pileus more ephemeral.

In form, colour, nitrous smell, spores and other microscopic details there was no difference. In some collections the spores were $7-9.5 \times 5-6 \mu$ and in others $6-7.5 \times 3.5-5 \mu$, also slightly amyloid. At most, it would seem to be a variety of *M. chlorophos*. Compare *M. margarita* Murrill and *M. umbilicata* Dennis (1951).

Mycena illuminans P. Henn. (Text-fig. 7)

Hedw. **42**, 309, 1903; Hoehnel, F. v., *S.B. Akad. Wiss. Wien*, **118**, Abt. 1, 279, 1909; (haud sensu Petch, *Ann. R. bot. Gard. Peradeniya*, **9**, 313, 1925, *M. chlorophos* (B. & C.)): Holtermann, *Mykol. Untersuch. aus d. Tropen*, Berlin, p. 106, 1898, as *Agaricus* sp.: Haneda, Y., *Kwagakunanyo*, **1**, 1939, f. 2, as *Mycena bambusa* Kawamura nom. nud.

Pileus 10–20 mm. wide, *convexo-campanulate*, *fuscous grey*, paler to the whitish margin, *sulcato-striate* to the disk, very minutely white pruinose. *Stem* 10–18 × 1 mm., translucent, *white*, minutely pruinose, the base discoid, 2 mm. wide and thick. *Gills* adnato-adnexed, 2–3 ranks, 15–16 primaries 2–3 mm. wide, rather thick and tough, often slightly veined at the base, *white*. *Flesh* thin, rather firm and tough, with a thin gelatinous hypodermal



Text-fig. 7. *M. illuminans*: a, cheilocystidia; b, pileocystidia; c, caulocystidia. × 500.

layer in the pileus: *smell nitrous*. *Spores* 10–11 μ wide, white, *subglobose*, thin-walled, smooth, with opalescent contents (contracting into a vitreous lump on drying), the apiculus 1 μ : reddish brown with Melzer's iodine. *Basidia* 30–38 × 11–13 μ , granular opalescent: sterigmata 4, 5 μ long: *paraphyses* none: subhymenium 12–20 μ thick, composed of 2–3 μ wide, subgelatinous, contiguous hyphae. *Cheilocystidia* 25–45 × 9–20 μ , as a sterile edge, colourless, thin-walled, subcylindric, clavate or ventricose, *verrucose-echinulate* over the apex with many small blunt conical processes –3 (–5) × 1–2 μ , not encrusted: *pleurocystidia* none. *Caulocystidia* as the cheilocystidia but more irregular, the processes –10 × 3 μ , or without processes, forming a distinct palisade on the stem: on the disk, 45–130 × 12–35 μ , fusiform, blunt, smooth without processes. *Pileocystidia* –50 × 10–30 μ , as the cheilocystidia. *Hyphae* clamped, inflating, slightly thick-walled: in the stem, longitudinal with cells 150–600 × 15–40 μ , the larger hyphae often secondarily septate (without clamps and with broad transverse septa), others little inflated with cells 30–350 × 2–5 μ , the walls –1.5 μ thick: in the pileus and gills, slightly agglutinated and somewhat pseudoparenchymatous, the cells 40–200 × 10–40 μ , not sec. septate, with abrupt transition to the subhymenium and to the gelatinous layer (*c.* 400 μ thick

when moist) composed of narrow hyphae ending in the palisade of pileocystidia: the outer hyphae of the flesh of the pileus and some of the gelatinous hyphae with brown sap.

On dead palm leaves and trunks, or on wood, solitary: Java, Singapore, Karimun Islands (Maro Island): apparently not common.

The large spores, warted cheilocystidia, and ascending gills distinguish this luminous species, as v. Hoehnel recognized it. He gave the spores as $8-9.5\ \mu$ wide, which may have referred to the contracted oily contents rather than to the very tenuous wall. The species is allied with *M. chlorophos*, but it is more substantially constructed, without paraphyses, and does not open the pileus so widely. The pileus and gills are luminous. This is, also, the species referred to in my paper on the basidium (Corner, 1947).

Section *Calodontes*

Mycena lux-coeli sp. nov. (Pl. 1, Text-fig. 8)

Pileus 15-25 mm. latus, conicus, saepe umbonatus, striatus, minute pruinosis, pallide purpureo-brunneus, margine crenulato. *Stipes* 20-45 $\times$ 1-1.5 mm., ex integro minute pruinosis, albus, basi villosus 1.5-2.5 mm. lato, subclavato, cavus. *Lamellae* adnatae, ascendentes, subdistantae, ordinibus 2-3, primariis 14-21, 2-2.5 mm. latis, interstitiis leniter venulosis, albae acie purpureo-brunneo. *Sporae* 8.5-12 $\times$ 6.5-9 μ , hyalinae, late ellipsoideae, obtusae, ut videtur non-amyloideae. *Basidia* 30-40 $\times$ 9.5-12 μ : sterigmata 4, 7-9 μ longa. *Cheilocystidia* 40-100 $\times$ 7-32 μ , acie sterili instructa, succo purpureo-brunneo, plus minus clavata v. ventricosa, saepe lobis multiformibus -25 $\times$ 2-6 μ obtusis praedita, tenuiter tunicata: pleurocystidiis nullis. *Pileocystidia* 13-25 $\times$ 3-9 μ , clavata, obtusa, tenuiter tunicata, succo purpureo-brunneo, primo dense instructa, pileo expanso disrupta, raro alia majora ut cheilocystidia. *Caulocystidia* ut pileocystidia, saepe decumbentia, apicem stipitis versus cheilocystidiis similiora. *Hyphae* nodoso-septata, inflata, tenuiter tunicata: stipite, -25 μ latae, septis latis transversis: pileo lamellisque -35 μ latae.

Ad corticem et lignum *Shiiae sieboldii* Makino: Japan (Ins. Hachijo, leg. Y. Haneda, 30 July 1953).

This is said to be common during June to August in Hachijo Island, which lies 24 hr. south from Tokyo by steamer. The gills and stem are bluish phosphorescent, but not the spores. The species seems close to *M. purpureofusca* but has larger spores and more lobed cystidia. The following is also allied.

Mycena noctilucens sp. nov. (Text-fig. 9)

Haneda, Y., *Kwagaku-nanyo*, 1, 1939, f. 5, ut *M. noctilucens* Kawamura nom. nud.

Pileus 2 cm. latus, plano-convexus, minute pruinosis v. velutinus, rubro-purpureus. *Stipes* 17 $\times$ 2 mm., ex integro minute pruinosis, cavus, basi abrupto, an concolori? *Lamellae* ventricoso-adnatae, latae, subdistantae, ordinibus 3, primariis 16, 5-7 mm. latis, haud reticulatis, albae, acie rubro-purpureo. *Sporae* 6-7.7 $\times$ 5.3-6.5 μ , hyalinae, ovoideo-lacrymiformes, ut videtur non-amyloideae. *Basidia* 20-27 $\times$ 7-9 μ : sterigmata 4, 6-7 μ longa. *Cheilocystidia* -40 $\times$ 6-14 μ , clavata v. subventricosa, tenuiter tunicata, succo fusco-rubro, raro lobata, acie sterili instructa. *Pleurocystidia* 30-55 $\times$ 8-18 μ numerosa, clavata v. ventricoso-subulata, processu apicali 2-5 μ lato, saepe succo fusco-rubro. *Pileocystidia* -30 $\times$ 5-12 μ , ut cheilocystidia sed saepe plus minus lobata, succo fusco-rubro v. guttulis concoloribus, plerumque dispersa. *Caulocystidia* -65 $\times$ 5-8.5 μ , subcylindrica, saepe et repetit lobata v. subdichotoma, obtusa, lobulis saepe subventricosis, succo fusco-rubro v. guttulis fusco-rubris, dense instructa. *Hyphae* nodoso-septatae, in stipite -25 μ latae: haud gelatinosae.

Ad radicem palmae: Micronesia, Ins. Yap et Palau, leg. Y. Haneda, Aug. 1937 (Yap-typus).

This species is common in Yap Island according to Haneda, but rare in Palau. I have not met with it in Malaya. It differs from the preceding, *M. lux-coeli*, in the smaller, solitary fruit-body with more expanded pileus, smaller spores and abundant pleurocystidia. It is also phosphorescent on the gills and stem with a similar pale blue light.



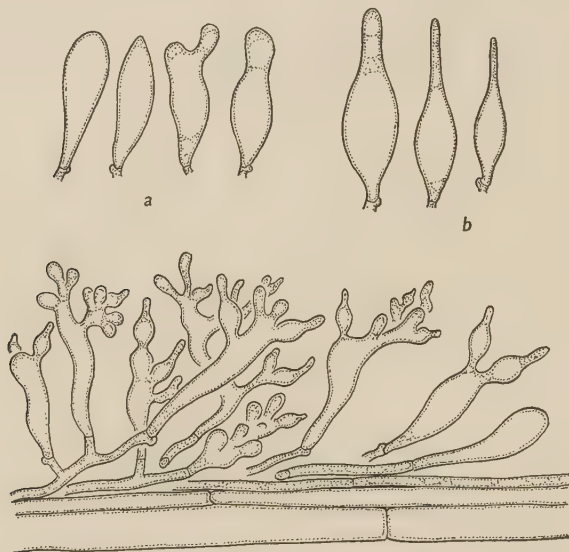
Text-fig. 8. *M. lux-coeli*, caulocystidia (above), cheilocystidia, and pileocystidia (below). $\times 500$.

Section *Glutinipedes*

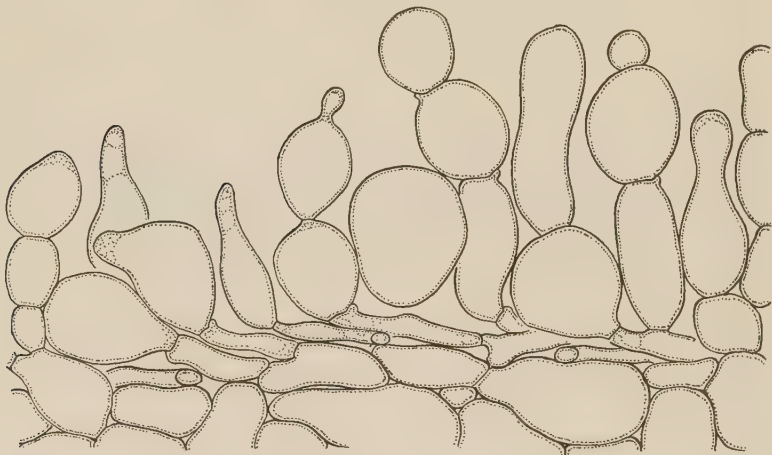
Mycena pruinoso-viscida sp. nov. (Text-fig. 10)

Pileus 3–13 mm. latus, plano-convexus, centro leniter depresso, striatus, albus v. flavidus, centro brunneolo v. subfusco, haud viscidus, sicco minute albido- v. fusco-pruinosis: margine primo incurvato. *Stipes* 1–3.5 cm. $\times$ 0.5–1 mm., cylindricus, basi abrupto, ex integro glutinosus, albus dein ochraceus v. brunnescens a basi apicem versus. *Lamellae* decurrentes, arcuatae, tenues, confertae, ordinibus 2–3, primariis 13–20, 1–2.5 mm. latis, albae dein cremæae v. subochraceae. *Sporae* 5–6.5 $\times$ 3–3.7 μ , hyalinae, ellipsoideae, aguttatae, leniter

cyaneo-amyloideae. *Basidia* $18-24 \times 5.5-6 \mu$: sterigmata 4. *Cheilocystidia* $20-35 \times 8-13 \mu$, clavata, raro lobata, hyalina, tenuiter tunicata, vix prominentia, acie sterili instructa: pleurocystidiis nullis. *Caulocystidia* $-70 (-100) \times 6-14 (-17) \mu$, plerumque clavata, interdum subventricosa v. subconica, saepe decumbentia, interdum apice proliferata, in



Text-fig. 9. *M. noctilucens*; a, cheilocystidia; b, pleurocystidia; and caulocystidia (below). $\times 500$.



Text-fig. 10. *M. pruinoso-viscida*, the surface of the pileus near its centre. $\times 500$.

glutine immersa, senescentia tunica brunnescentia. *Pilei* superficies cellulis subglobosis, $30-60 \times 10-40 \mu$, succo umbrino instructa, pilei centro cellulis 2-3 seriatim conjunctis, marginem versus dispersis ut pileocystidiis subglobosis v. clavatis. *Hyphae* nodoso-septatae: in stipite -20μ latae, cellulis -400μ longis, septis transversis latis, ad superficiem angustae, tunicis gelatinosis, aetate tunicis brunnescentibus: in pileo superne pseudoparenchymaticae, cellulis -35μ latis, inferne plus minus longitudinaliter intertextis ut in lamellis: in subhymenio, $15-20 \mu$ crasso, angustae, intertextae, subgelatinosae.

Ad lignum emortuum in silvis, *caespitosa*, inodora: Malaya (Pahang, Fraser's Hill, c. 1300 m. alt., 23 Sept. 1940, Corner 849, *typus*: Johore, pr. Mawai, 11 Nov. 1940, Corner 850).

var. **rabaulensis** var. nov.

Haneda, Y., *Kwagaku-nanyo*, 4, 52-53, 1942, ut *Omphalia* sp.

Sporae $6.5-8.3 \times 3.5-4 \mu$: *basidia* c. $30 \times 6 \mu$: *cheilocystidia* $30-42 \times 8-11 \mu$, prominentia: caulocystidia magis evoluta: cellulae subglobosae ad superficiem pilei sparsae v. nullae.

Ad lignum emortuum caespitosa, Rabaul, Nova Britannia, Mart. 1940, leg. Y. Haneda.

This species is close to *M. rorida*, but the colour of the fruit-body is clearer, the habit is densely caespitose, and the covering of subglobose cells on the pileus is better developed, at least in the typical Malayan collections of *M. pruinoso-viscida*. The caulocystidia are, also, better developed, and the gills are more crowded. The spore-deposit also seems to be luminous, as figured by Haneda, but the same problem may arise here as Josseland has shown in *M. rorida* (1953). In var. *rabaulensis* the gills were slightly wider, -3 mm. , and the interstices were slightly veined. Young fruit-bodies of the Malayan collections had the pileus covered by the short rows of subglobose cells, representing a better developed state than that in *M. rorida*, but these cells were mostly absent from the Rabaul specimens.

***Mycena sublucens* sp. nov.** (Text-fig. 11)

Pileus -10 mm. latus, convexus, *subumbilicatus*, striatus, subpruinosis, *fusco-pallidus*, margine primo incurvato, carne centro crassiuscula. *Stipes* $10-15 \times 1.5-2 \text{ mm.}$, cylindricus, *pruinosis*, basi abrupto, *viscidus*, *albidus*. *Lamellae* breviter decurrentes, *angustae*, *confertae*, *reticulato-connexae*, ordinibus 3-4, primariis $16-25$, $1-1.5 \text{ mm.}$ latis, *albae*. *Sporae* $6-7 \times 5-5.7 \mu$, hyalinae, ovoideo-ellipsoideae, ut videtur non-amyloideae. *Basidia* $25-33 \times 7 \mu$: sterigmata 4. *Cheilocystidia* $30-50 \times 8-12 \mu$, clavata v. subventricosa, ad apicem lobulis diversis $-9 \times 1-2.5 \mu$ praedita, tenuiter tunicata, acie sterili instructa: pleurocystidiis nullis. *Caulocystidia* ut cheilocystidia sed saepe magis lobata, copiosa. *Pileocystidia* $-95 \times 8-18 \mu$, subclavata, levia, tenuiter tunicata, plerumque decumbentia. *Hyphae* nodoso-septatae: in stipite -25μ latae, ad superficiem angustae et gelatinosae: in pileo -15μ latae, haud pseudoparenchymaticae, ad superficiem angustae in strato gelatinoso $50-120 \mu$ crasso immersae.

Ad radicem bambusae: Amboina, leg. Y. Haneda, Mart. 1942.

This rather indistinctive *Mycena* has much in common with *M. rorida*, but the absence of subglobose cells from the surface of the pileus and the presence of a gelatinous hypodermis will prevent confusion. Moreover, the stipe and gills are luminous, according to Haneda, not the spores or the substratum around the base of the fruit-body. I have not collected the species in Malaya, and Haneda has not found it in other places than Amboina.

Section *Rigidipedes*

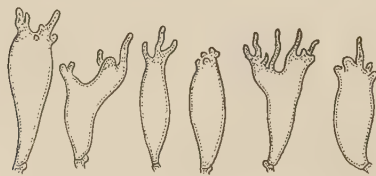
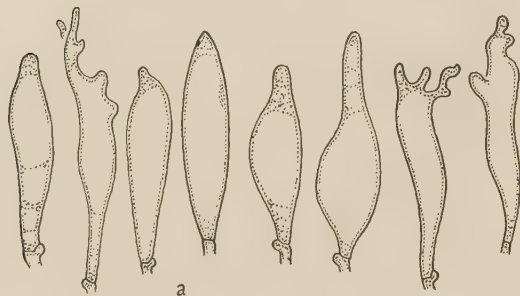
***Mycena manipularis* (Berk.) Métrod** (Text-fig. 12)

Prodr. Fl. Mycol. de Madagascar, III, Les Mycènes, 87, 1949, f. 51.

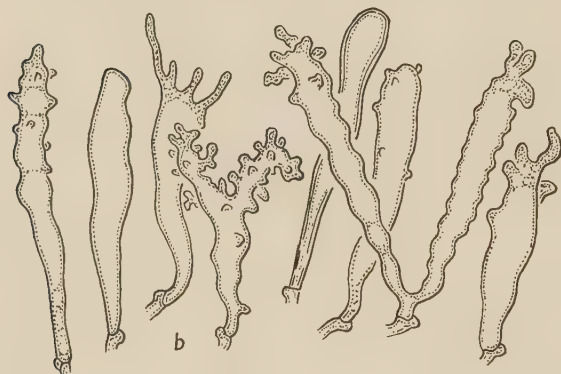
Basinym: *Favolus manipularis* Berk., *Hooker's J. Bot.* 6, 229, 1854.

Syn.: *Poromyceia pallescens* Boedijn, *Bull. Jard. bot. Buitenz.*, Ser. III, 16, 398, 1940, f. 8.

P. manipularis (Berk.) Heim, *Rev. Mycol., Paris*, 10, 35, 1945, f. 21-23, pl. III, f. 1-3.

Text-fig. 11. *M. sublucens*, cheilocystidia. $\times 500$.

a



b



c

Text-fig. 12. *M. manipularis*; a, cheilocystidia; b, caulocystidia; c, pileocystidia, $\times 500$. Spores, $\times 1000$.

P. Hanedai Y. Kobayasi, J. Hattori bot. Lab. n. 5, 1, 1951, f. 1 a: Haneda, Y., *Kwagaku-nanyo*, 1, 1939, f. 8A-C, as *Polyporus Hanedai* Kawamura nom. nud.

Filoboletus manipularis (Berk.) Singer, *Lloydia*, 8, 215, 1945: Dennis, R. W. G., *Kew Bull.* p. 325, 1952.

Pileus 0.5–3.5 cm. wide, convex or conico-campanulate, *umbonate*, then plane, *hygrophanous*, somewhat translucent, *tesselately sulcate* from the tubes, finely white pruinose, then subviscid, *sepia brown, fuscous or fawn when young, paler on expansion to greyish or nearly white, often yellowish with age or discolouring pink, then brick-red* from the centre outwards: margin rather strongly incurved at first. *Stem* 0.4–6 cm. $\times$ 0.5–2.5 mm., cylindric or thickened –3.5 mm. at the abrupt or slightly dilated base, hollow, often flattened, brittle, *wholly white pruinose*, becoming smooth, *paler concolorous*, often discolouring brownish, pinkish or reddish from the base. *Tubes* 1.5–5 mm. long, *adnate* or with a slight decurrent tooth, *in regular radial rows*, with shorter rows inserted, paler concolorous, waxy: *pores angular, large*, 0.5–1 mm. wide, 12–18 primary pores, 5–10 pores along the main radii, *white then pale cream*. *Flesh* 0.5–2.5 mm. thick in the centre, thin over the limb, soft, waxy, hygrophanous, pallid: smell rather waxy: *greenish phosphorescent*, especially in the lower part of the stem. *Spores* 6–8.5 $\times$ 4–5.5 μ , white, smooth, broadly and bluntly ellipsoid, rarely pip-shaped, opalescent, aguttate, bluish amyloid. *Basidia* 20–28 $\times$ 6–8 μ , short, clavate: sterigmata 4, 6–8 μ long, stout. *Cheilocystidia* 35–75 $\times$ 7–15 μ , subclavate to subventricose or broadly lanceolate, the apex acute, often shortly lobed or processed, thin-walled, colourless, forming a sterile edge but soon collapsing in the expanded pileus: *pleurocystidia* none. *Caulocystidia* –95 $\times$ 5–16 μ , elongating –150 μ in the lower part of the stem and with transitions to emergent hyphae 3–5 μ wide, more or less clavate or lanceolate, varying subventricose, *mostly with irregular lobes* –15 $\times$ 2–4 μ , very variable, the walls sometimes slightly thickened. *Pileocystidia* similar to the caulocystidia, collapsing on expansion of the pileus and giving the subviscid surface. *Hyphae* clamped, much inflated except at the surface of the stem and pileus and in the subhymenium, the walls thin or slightly thickened: the cells 40–250 $\times$ 8–50 μ in the pileus and tubes, –400 $\times$ 25 μ in the stem, with numerous uninflated hyphae, a few laticiferous: almost pseudoparenchymatous below the surface of the pileus: the walls subgelatinous in the subhymenium.

On sticks, fallen trunks and stumps in the forest, caespitose: Tropical Asia (Ceylon, Malaya, Sumatra, Borneo, Krakatau, Karimun Islands, Philippine Island, Ponapé), New Guinea, New Caledonia, Australia, Madagascar, Venezuela, Japan.

var. **microporus** Kawamura ex Corner, var. nov.

Haneda, Y., *Kwagaku-nanyo*, 4, 226, f. E, and 227 f. A–B, 1942; as *Polyporus microporus* Kawamura nom. nud.

Poris minoribus 0.3–0.5 mm. latis, magis numerosis, primariis 16–22, 9–17 radiato-seriatim: *sporis* minoribus 5–6.5 $\times$ 4–5.3 μ , an maturis?: *hyphis* in dissepimentis tuborum magis gelatinosis: *cheilocystidiis* minoribus, –35 $\times$ 10 μ , magis lobatis: *caulocystidiis* multum lobatis.

Ad lignum: Ponapé Island (*typus*: Haneda n. 5, in herb. universit. cantabr.).

This polyporoid *Mycena* is probably world-wide in the tropics and sub-tropics, though not reported from the African mainland. It is now fairly well known through the researches of Boedijn, Heim and Singer, who give additional synonyms. Haneda was the first, however, to discover its luminosity, which he showed to me in Singapore, and which has been confirmed by Kobayasi: the whole fruit-body, especially the lower part of the stem, is greenish phosphorescent. The species is common in the Malaysian region, but I have not met with another at all closely related. I prefer to follow Métrod in placing the species in *Mycena*. Both *Filoboletus* and *Poromyцена* are form-genera for various polyporoid derivatives of *Mycena*, possibly also of *Omphalia*, and the alliance of *M. manipularis* is not with *Filoboletus mycenoides*. There is still some uncertainty about the cystidia, though Boedijn has described them. There may also appear discrepancies in the colour of the fruit-body, which changes much during its life. I have, therefore, described the fungus as it occurs in Malaya.

The fruit-bodies are gymnocarpic. At an early stage the surfaces of pileus and stem develop a loose palisade of irregular cystidia, which persist until expansion of the fruit-body. The pileocystidia then disintegrate, giving the slightly viscid surface, though a few with somewhat thickened walls may persist. The palisade on the stem becomes disrupted, by the elongation of the stem, into the pruina of caulocystidia. Similar, but more regular, cystidia develop on the edges of the tubes, but they also collapse and mucify on expansion of the pileus.

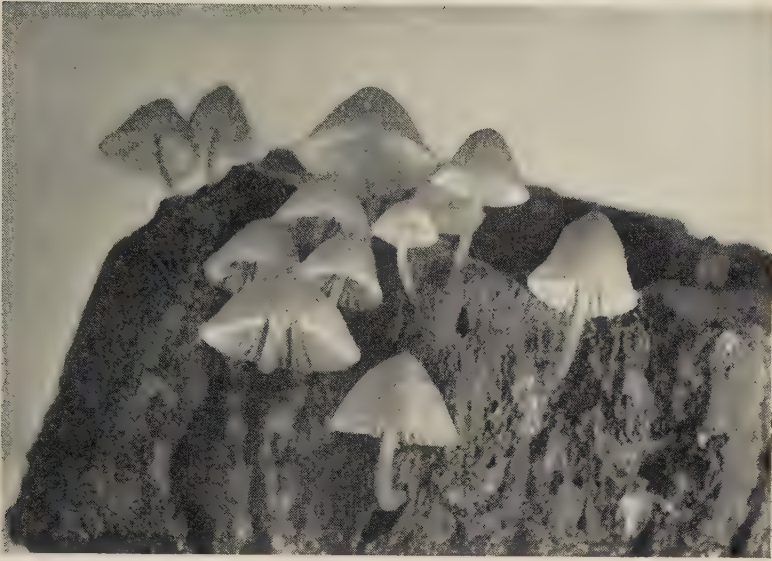
In 1930 and 1934, I studied the development of the fruit-bodies on logs of wood which I brought to the Singapore Botanic Gardens from the forest in Johore. The primordia are recognizable as minute flecks c. 0.2 mm. wide, when they are about 24 hr. old. They take 4-5 days (about 100 hr.) before they begin to expand. This process takes about 2 more days for completion, and the full-grown fruit-bodies last for 2-6 days sporing. Thus the fruit-bodies are remarkably long-lived for a tropical *Mycena* and slow in their expansion. In ageing, some fruit-bodies turn yellowish, but most become pinkish, and then, more or less brick-red from the centre of the pileus outward and from the base of the stem upwards: the discoloration may be the effect of moulds. Under wild conditions the fruit-bodies are soon attacked by fungus-gnats, which hasten their decay. The following is a typical life-history:

Diameter of the pileus/overall height in mm. at 8 a.m. on successive days, Singapore 1930

Days	1	2	3	4	5	6	7	8	13
	0.2/0.2	0.4/0.4	0.8/1	1.5/2	3.3/5	7/9	16/20	19/21	Dead 4 p.m.
						expansion			

REFERENCES

- CORNER, E. J. H. (1947). Variation in the size and shape of spores, basidia and cystidia in Basidiomycetes. *New Phytol.* **46**, 195-228.
 CORNER, E. J. H. (1950). Descriptions of two luminous tropical agarics (*Dictyopanus* and *Mycena*). *Mycologia*, **42**, 423-431.
 DENNIS, R. W. G. (1951). Some Agaricaceae of Trinidad and Venezuela. *Leucosporae*: Part 1. *Trans. Brit. mycol. Soc.* **34**, 411-482.



- OSSERAND, M. (1953). Sur la luminescence de *Mycena rorida* en Europe Occidentale. *Bull. mens. Soc. linn. Lyon*, **22**, 99-102.
- KOBAYASI, Y. (1937). Several luminous Mycomycetes from Bonin Islands. *Bull. biogeogr. Soc. Japan*, **7**, 1-7.
- VASSINK, E. C. (1948). Observations on the luminescence in Fungi, 1, including a critical review of the species mentioned as luminescent in literature. *Rec. Trav. bot. néerl.* **41**, 150-212.

EXPLANATION OF PLATE 9

Mycena lux-coeli from Hachijo Island, Japan, photographed by Y. Haneda in nature and by its own light in the dark room (F. 3·5, exposure 50 min.). About $\frac{2}{3}$ nat. size.

(Accepted for publication 4 November 1953)

CERIOSPOROPSIS HALIMA LINDER AND CERIOSPOROPSIS CAMBRENSIS SP. NOV.: TWO MARINE PYRENOMYCETES ON WOOD

By IRENE M. WILSON

University College of Wales, Aberystwyth

(With 23 Text-figures)

British collections of *Ceriosporopsis halima* have enabled some details to be added to Linder's original description of this species, notably the presence of a long perithecial neck and an account of the asci and ascospores.

C. cambrensis sp. nov. is described. It differs from *C. halima* mainly in its larger perithecia and spores and its paler colour.

The development and structure of the stromatic perithecia and of the asci of these two species indicate that they are allied to the Pseudosphaeriales.

In the succession of fungi on wood immersed in the sea, these two species generally follow *Halophiobolus opacus* Linder, *Ceriosporopsis halima* slightly preceding *C. cambrensis* if both occur together, and they may in turn be succeeded by other fungi, but, if they are on wood in the intertidal region near low water mark, they encourage and facilitate the entry of the far more destructive, boring crustaceans and it is in this that any importance lies which they may possess as agents in the destruction of timber.

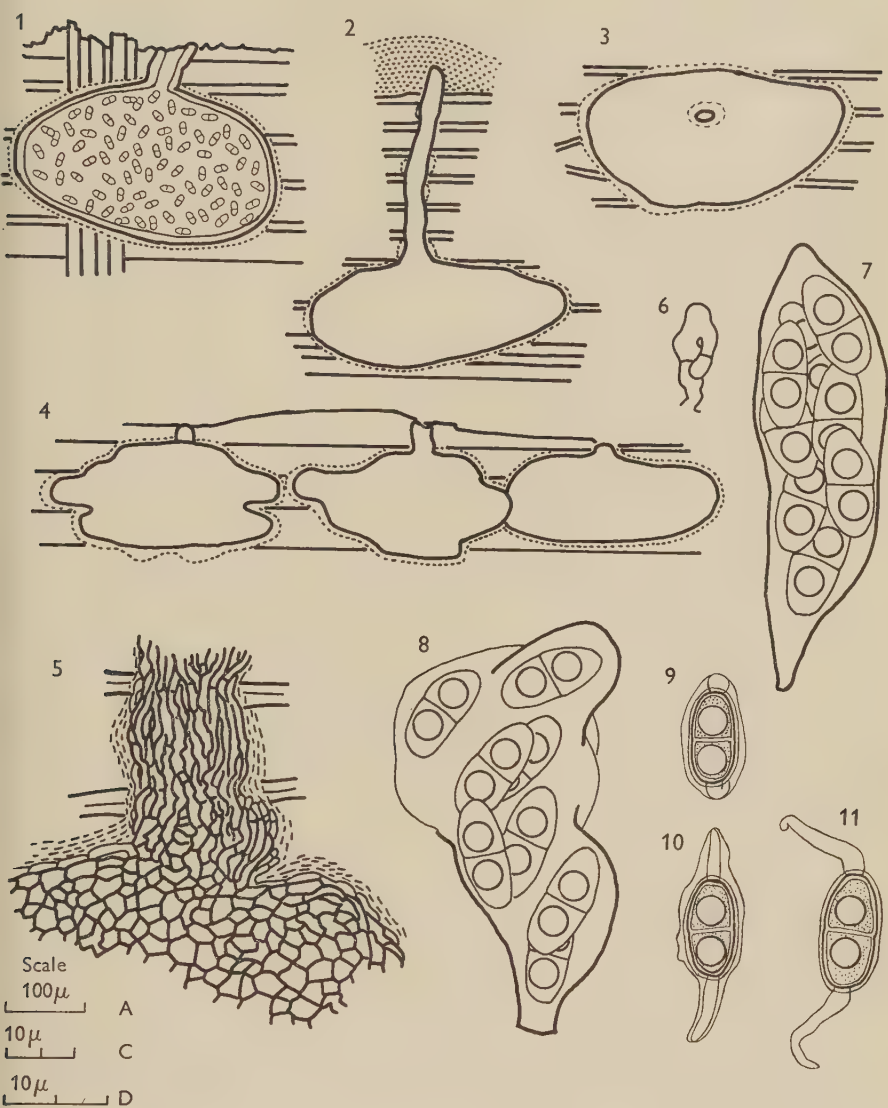
Ceriosporopsis halima was described by Linder (Barghoorn & Linder, 1944) as the type of a new genus and species. The genus *Ceriosporopsis* was distinguished from *Ceriospora*, which it closely resembles, by the irregular shape of the membranous collapsing perithecia, the deliquescent asci and the deliquescent or dehiscent appendages of the ascospores. *Ceriosporopsis halima* was found on wood which was completely submerged in the sea or in brackish water at several localities in New Hampshire, Massachusetts and Connecticut, U.S.A. It was described from fully mature specimens in which the spores were already free inside the perithecium, but it could be inferred that these spores were ascospores, and not conidia, by their frequent occurrence in clusters of eight and by the absence of a hilum.

In the course of some investigations on marine fungi along the southern half of the west coast of Wales, nine collections of *C. halima* have been made, some of which have allowed the observation of earlier stages than were available to Linder. Two collections have been made of another species of *Ceriosporopsis* which differs from the type species *C. halima* in its much larger perithecia and its larger spores. This species, which appears to be new, is described later in this paper as *C. cambrensis* sp. nov.

***Ceriosporopsis halima* Linder (Figs. 1-11)**

Locality and habitat

The two main localities on the west coast of Wales for the British collections were Watwick Bay, Pembrokeshire and Aberystwyth, Cardiganshire (Wilson, 1951).



Figs. 1-11. *Ceriosporopsis halima* Linder. Figs. 1-3. Perithecia on coniferous woods. Neck complete in Fig. 2 and projecting into sediment on surface of wood; neck worn down in Figs. 1 and 3. Surface view in Fig. 3. Fig. 4. Perithecia in alder; necks worn down to varying degrees. Dotted line round perithecia in Figs. 1-4 represents crushed wood. Fig. 5. Surface view of upper part of venter and lower part of neck. (Crushed wood round perithecia shown diagrammatically.) Fig. 6. Crozier. Fig. 7. Ascus with 8 ascospores. Fig. 8. Ascus with outer layer of wall broken and inner protruding. Both layers become mucilaginous. Fig. 9. Detail of ascospore from an ascus as in Fig. 7. Appendages are folded against side of ascospore and whole spore is enclosed in mucilage. Fig. 10. Ascospore as it appears while still within perithecia as in Fig. 1. Appendages partially extended but still enclosed in mucilage. Fig. 11. Ascospore in sea-water, after release from perithecia. Mucilage envelope has disappeared. Figs. 1-4: scale A; Fig. 5: scale C; Figs. 6-11: scale D.

All the specimens were found on wood, eight on coniferous woods, including species of pine and spruce, and one on alder. Only one piece of wood was completely submerged near mean low water mark of spring tides. Seven suffered varying degrees of exposure in the intertidal area from near mean low water mark of neap tides to positions higher up the shore among plants of *Fucus vesiculosus*, or even in the *Ascophyllum nodosum* zone in areas in which the latter was the higher up the shore. One specimen was on driftwood. A common factor of all these habitats was that the wood remained constantly wet; in the intertidal region *Ceriosporopsis halima* developed only on wood which was not directly exposed to the sun or which was shaded and kept moist either by overhanging fronds of the larger brown algae or by being partially buried in sand.

On test blocks of freshly sawn timber fastened mainly within a fairly short range above and below M.L.W.M.N., *C. halima* with ripe perithecia and ascospores developed within $4\frac{1}{2}$ months after exposure of the wood in the sea. It often followed closely after *Halophiobolus opacus* Linder which, after fruiting, could still be recognized in the wood by its characteristic chlamydospores. In one specimen, higher up the shore, *Remispora maritima* Linder and *Peritrichospora integra* Linder were associated with *Halophiobolus opacus* and preceded *Ceriosporopsis halima*. In sheltered conditions *C. halima* continued to fruit on the same block for $3\frac{1}{2}$ years. After *C. halima*, *C. cambrensis* may develop, or two, as yet undescribed, species of *Halophiobolus*, one characterized by its reddish perithecia and the other by the round inflated appendages of the ascospores. When the wood blocks were near the ground near M.L.W.M.N., the end of the succession of fungi was sometimes brought about by the much more destructive activities of wood-boring crustaceans; *Chelura terebrans* was the species seen, but, from the appearance of the wood, this probably followed an attack by *Limnoria lignorum*.

Structure and development of the perithecium

The British material allows one important addition to be made to Linder's description of the perithecium, namely the presence of a long neck (Fig. 2). As the wood, in which the perithecium is at first completely immersed, is extensively permeated by the hyphae of the fungus, it is in a soft and rotten condition and is easily eroded by the action of the sea. Many older perithecia are therefore seen in the condition depicted in Figs. 1, 3 and 4 with necks worn down to the level of the wood until finally only a small papilla remains. It is this end condition which was described by Linder (Barghoorn & Linder, 1944).

Some American material of *Ceriosporopsis halima* Linder which I examined in 1950 by kind permission of the late Dr W. Lawrence White of the Farlow Herbarium and Library, Cambridge, Massachusetts, had mature perithecia in the condition described by Linder and also younger perithecia containing the characteristic spores of *C. halima* and with long necks as in the British material. It therefore appears that the long neck is a characteristic feature of the species.

The size and shape of the perithecia vary considerably with the type of wood in which they develop, and according to whether they arise in the

spring or autumn wood. In alder (Fig. 4) they are small and very irregular in outline, the perithecia expanding into the natural cavities of the xylem elements and being constricted by the walls. Some accommodation to the xylem elements is also found on coniferous woods, but, on the whole, the perithecia in these softer woods are larger and more ovoid in form (Figs. 1-3). They are also less constricted in depth when formed in spring wood than in autumn wood. In all cases there is a zone of brown, crushed wood round the perithecium (dotted in Figs. 1-5), partially corroded by extra-matrical enzymes and extensively penetrated by hyphae from the perithecial wall.

The development of the perithecium is essentially similar to that of *C. cambrensis* (Figs. 12-15), but there appear to be fewer large (sexual ?) cells in the young stroma of *C. halima*.

Asci and ascospores

The asci arise from croziers (Fig. 6). They are 8-spored (Figs. 7, 8). The wall is 2-layered and is of uniform thickness throughout. Though tough, it is not particularly thick as is, for example, the wall in *Pleospora herbarum*. There is no apical pore. When ripe, the ascus swells enormously laterally, the outer layer breaks, often near the middle of the ascus, and the inner layer bulges out (Fig. 8), but soon both layers become so mucilaginous that they can no longer be distinguished.

The hyaline 2-celled spores have one large oil drop in each cell. The wall is 2-layered and thicker than the ascus wall, the outer layer being clear and colourless. At first the appendages, one at each end, lie against the side of the spore enclosed in a compact, ovoid mass of mucilage (Fig. 9). While still within the perithecium (Fig. 1) the spores remain surrounded with mucilage as in Figs. 9 or 10, but, when they ooze out of the ostiole into the sea water, the mucilage envelope swells and eventually disappears and the appendages can then become more fully extended (Fig. 11). Since the appendages are very elastic and readily adhere to any solid object, even glass, they can be stretched out to a considerable length. Old spores may be seen without appendages and faintly coloured as described by Linder.

Dimensions of perithecia asci and ascospores

The measurements of the American and British material are compared in Table 1. There is no doubt that they represent the same species.

Table 1. *Comparison of measurements of American and British material of Ceriosporopsis halima Linder*

	(Measurements in μ .)	
	American*	British
Perithecium	175-400 $\times$ 90-300	167-404 $\times$ 90-257
Length of neck	—	Up to 262
Ascus	—	80-90 $\times$ 21-26
Ascospores	22.5-26-(35) $\times$ 8-10	21-26 $\times$ 8-10

* From Barghoorn & Linder (1944).

Germination of spores and cultures

In contrast with most terrestrial fungi there appears to be no seasonal fruiting period. Ascospores have been found all the year round. On release they germinate readily without a resting period, for example, within 3 days on sea-water agar at room temperature. Cultures grow slowly on 0.5 % malt agar made up with sea water. The conidial stage, *Helicoma salinum* (Barghoorn & Linder, 1944) has not yet been observed on the few media used so far.

Barghoorn (Barghoorn & Linder, 1944) has studied the rate of radial growth of the mycelium of *Ceriosporopsis halima* in a series of sea-water agar cultures, each containing, separately, a different organic substance. He found that the greatest radial growth occurred when pectin was the substance added, followed by starch, maltose, cellulose and xylose (bracketed equal), wood flour, galactose and asparagine, in that order. These facts will be related to other observations at the end of this paper.

***Ceriosporopsis cambrensis* sp.nov. (Figs. 12-23)**

Perithecia gregaria, singula vel in series breves secus ligni strias contiguo ordinata; primum omnino immersa, denique semiimmersa, in superficiem ligni erosam basi insculpta; dorso-ventraliter ellipsoidea, 577-862 μ longa, $\times$ 260-300 μ lata, $\times$ 276-548 μ alta; membranacea, externe pallido colorata, sed vestimento brunneo cellularum ligni disjectarum circumdata; superne collo tenui, externe brunneo, aliquantulum excentrico, ad 880 μ longo, gradatim tamen attrito praedita.

Asci clavati, sessiles, nonnihil asymmetrici, aequaliter bitunicati, poro apicali carentes 100-130 $\times$ 24-26 μ , 8-spores, in maturitate mucosi.

Paraphyses verae nullae.

Ascospores primum oblique monostichae, subcylindratae, 0-septatae, mox inordinatae, ellipsoideae, medio 1-septatae, vix constrictae, cellula quisque guttulam oleosam includens, utrimque obtusae (seu vero paululum truncatae) ac appendicula singula ornatae, hyalinae, 29-31.5 $\times$ 10.5-14.5 μ (sine muco et appendiculis); appendiculae primum recurvatae, dein subrectae, hyalinae, 36 $\times$ 3.6 μ , mox deciduae vel deliquiscentes.

Hab. In ligno *Alni glutinosae* aqua marina diu madefacto; Aberystwyth, Cambriae; 20. iii. 1950 (Herb. I.M.I. 53595).

Obs. A *Ceriosporopsis halima* peritheciis ascosporisque majoribus, pariete peritheciali pallidiore sat differt.

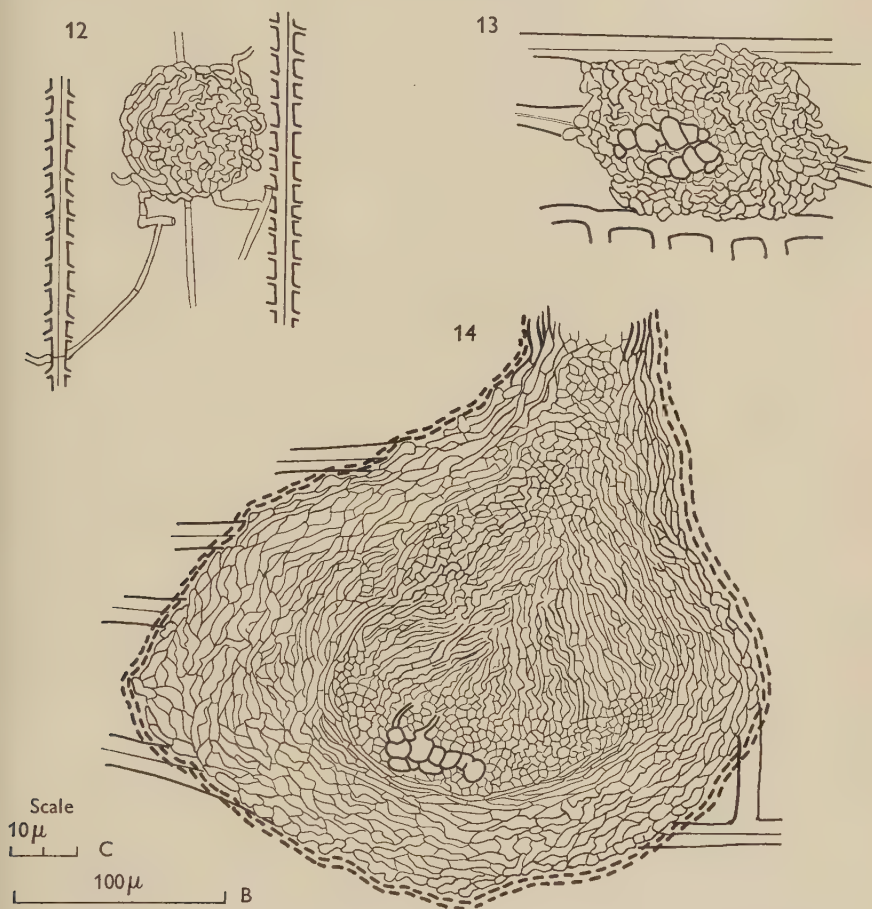
Perithecia gregarious, arranged singly, or contiguously in short series, along the elements of the wood; at first completely immersed, becoming exposed by the erosion of the wood; dorsiventrally ellipsoid, 577-862 μ long $\times$ 260-300 μ broad $\times$ 276-548 μ high, membranous, externally palely coloured but surrounded by a brown layer of crushed elements of wood; provided above with a slender, externally brown neck, slightly excentric, up to 880 μ long, but gradually worn away.

Asci clavate, sessile, somewhat asymmetric, wall 2-layered and of equal thickness throughout, lacking an apical pore, 110-130 $\times$ 24-26 μ , 8-spored, mucilaginous at maturity.

True paraphyses lacking, but interthecial stromal elements present among asci.

Ascospores at first obliquely monostichous, subcylindrical and aseptate, soon irregularly arranged and ellipsoid, then 1-septate with median septum and very slightly constricted, each cell containing an oil drop, each

end of the spore obtuse (or if truncate only slightly so) and furnished with a single appendage at each end, spores hyaline, $29-31.5 \times 10.5-14.5 \mu$ (without mucous envelope and appendages); appendages at first curved in, afterwards more or less straight, hyaline, $36 \times 3.6 \mu$, soon deciduous or deliquescent.



Figs. 12-14. *Ceriosporopsis cambrensis* sp.nov. Fig. 12. Young stroma in cavity of tracheid. No sex cells. Fig. 13. Slightly older stroma in which sexual cells have been differentiated. Fig. 14. Stroma differentiated into an outer layer which is beginning to form the neck, and a central tissue, pseudoparenchymatous at top and bottom and hypha-like in centre. Primary ascogenous hyphae arising from sexual cells. Rim of dark-coloured crushed wood round stroma. Figs. 12, 13: scale C; Fig. 14: scale B.

Habitat. In wood of *Alnus glutinosa* kept wet for a long time in the sea; Aberystwyth, Wales; 20 March 1950 (Herb. I.M.I. 53595).

Differs from *Ceriosporopsis halima* in its larger perithecia and ascospores, and in the paler perithecial wall.

The type material of *Ceriosporopsis cambrensis* sp.nov. has been deposited in the herbarium of the Commonwealth Mycological Institute, Kew.

Locality, habitat and effect on substrate

The type material of this new species was found on driftwood floating in the sea at Aberystwyth on 20 March 1950. On this same piece of alder wood there were old perithecia of *C. halima*, some still with asci and spores, and immature perithecia of a species of *Halophiobolus* with reddish perithecia.

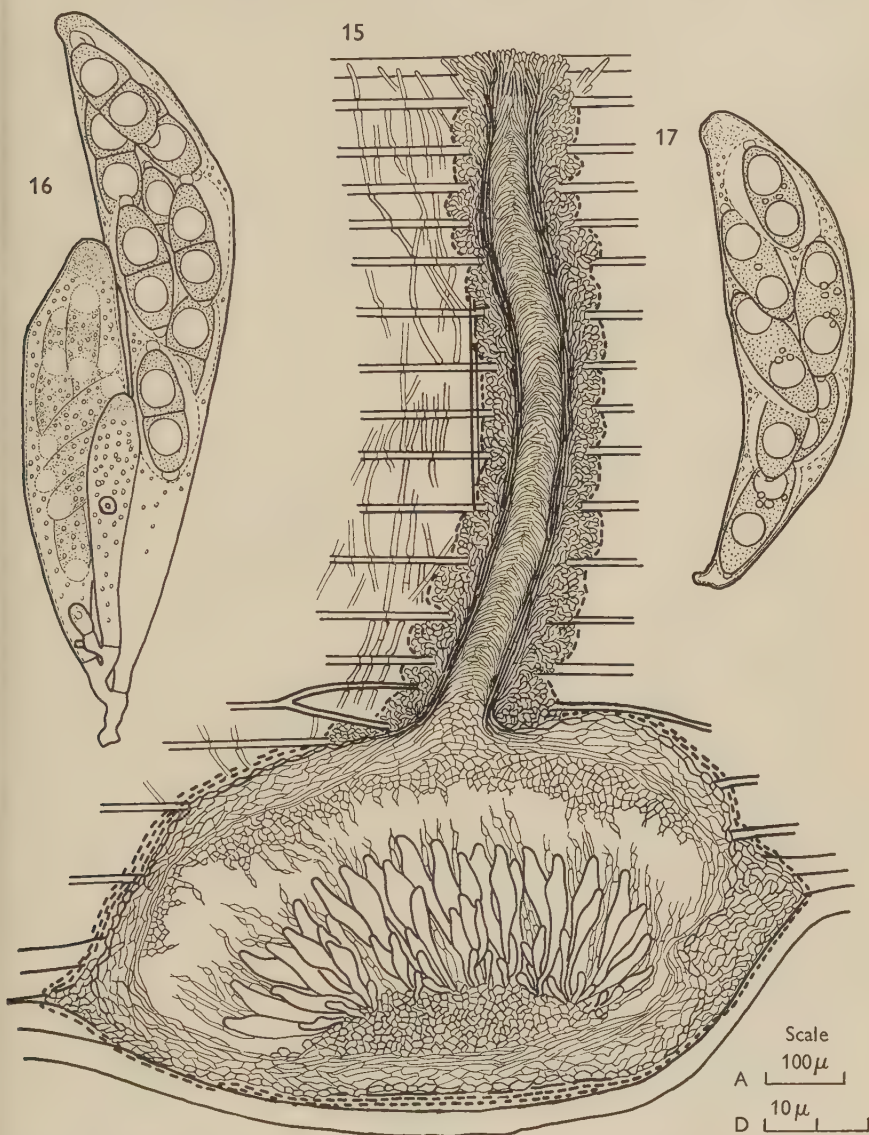
Subsequently, in 1953, *Ceriosporopsis cambrensis* was found on a block of wood (Douglas fir) embedded in a concrete block which had fallen out of the wall surrounding the Children's Pool at Aberystwyth and was lying, partly immersed, in a rock pool among plants of *Fucus serratus*, *Laminaria digitata* and *L. saccharina*, well shaded from direct sunlight. It is not known how long the wood was exposed to sea water through cracks in the concrete before it fell out of the wall, but within a month of doing so the part immersed in the pool bore ripe perithecia of *Halophiobolus opacus*, and, in the same part of the wood, there were very young perithecia (Fig. 12) of *Ceriosporopsis cambrensis*. The *Halophiobolus opacus* then ceased to fruit but remained conspicuously present in the wood in the form of chlamydospores. In a further 2 months the perithecia of *Ceriosporopsis cambrensis* were fully mature. The perithecia occupied a depth of about one annual ring and the wood surrounding them was conspicuously riddled by coloured hyphae which ran through, and obliquely across, the tracheids and formed bore-holes through the walls (Fig. 15). The mycelium did not penetrate below the next annual ring. The medullary rays also contained hyphae, singly or in strands, within the cells and occasionally also in the place of the middle lamella. It is certain that wood infected by *Halophiobolus opacus* and *Ceriosporopsis cambrensis* is more quickly eroded by the sea than similar uninfected wood. While the substrate and the necks of the first perithecia were being worn away, a second crop of perithecia was already well advanced at a slightly lower level and these were mature a fortnight later. Only four successive crops of perithecia were formed before the wood was attacked by *Limnoria lignorum*, the Gribble, a boring crustacean, which could be seen sheltering in the old, empty perithecial cavities of *Ceriosporopsis*. Soon the wood began to be riddled by the typical perforated galleries of *Limnoria* which run parallel to the surface of the substrate. The outside of the wood was thus made very friable. At the time of writing, 12 weeks after the *Limnoria* attack began, the fungus was still maintaining itself, though more feebly, and mycelium and young perithecia were seen in the wood below the level of the tunnels.

Development of the ascocarp

The earlier part of the sequence described here was followed on the material on Douglas fir, the latter part on alder also.

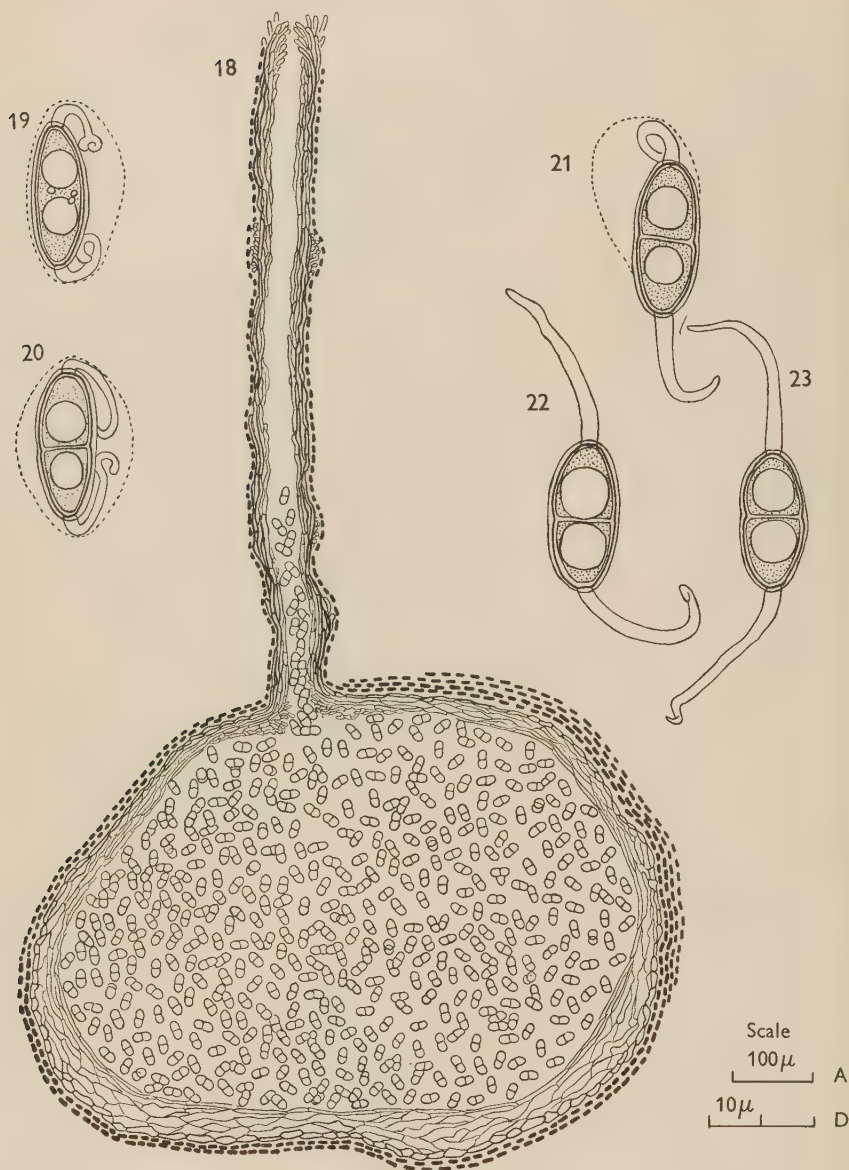
The earliest stage observed consists of a small knot of pseudoparenchymatous tissue, interpreted as a stroma since it arises before the sex organs (Miller, 1928). It is of a pale colour with light shining walls lying within the cavity of a tracheid (Fig. 12). In section, at a slightly later stage (Fig. 13), some very large rounded cells, which probably represent the sexual apparatus, can be seen within the stroma. It was not deter-

mined with certainty whether these were in the form of a single ascogonial coil or whether two separate chains of cells were present.



Figs. 15-17. *Ceriosporopsis cambrensis* sp. nov. Fig. 15. Stromatic perithecium with asci at all stages of development on Douglas fir. Crushed wood round outside represented digrammatically. Fig. 16. Group of four asci at different stages of development formed by lateral proliferation. Fig. 17. Immature ascus with seven of the eight spores, not yet septate, but appendages and mucilage envelope present. Fig. 15: scale A; Figs. 16, 17: scale D.

At the stage shown in Fig. 14, the stroma has expanded considerably and now extends across the cavities of several tracheids whose walls it has crushed but only partially absorbed in its growth. Two distinct regions can



Figs. 18–23. *Ceriosporopsis cambrensis* sp. nov. Fig. 18. Mature stromatic perithecium on alder containing ascospores and mucilage. Neck has not reached surface of wood in which it is immersed. Fig. 19. Immature, aseptate spore with appendages and mucilage envelope. Fig. 20. Mature, septate spore still in mucilage envelope. Fig. 21. Mucilage slightly displaced to release one appendage. Figs. 22, 23. Mature spores without mucilage envelope and with appendages fully extended (all spores drawn mounted in sea-water). Fig. 18: scale A; Figs. 19–23: scale D.

be distinguished, the wall and the central tissue. The latter contains, near its base, the large cells which probably represent the sexual apparatus. The compact, pseudoparenchymatous tissue of the central region is particularly deep at the bottom and at the top, where it forms a dome-shaped projection towards the base of the neck, but the central part is stretched out by the expansion of the whole central region to form paraphysis-like hyphae, the pseudoparaphyses (in the sense of Miller (1949), not Petrak). Primary ascogenous hyphae arising from one, or possibly more, of the sexual cells are present at this stage.

The inner part of the wall consists of hyaline cells which are much stretched and flattened; their condition is an indication of the expansion of the central tissues. The outer hyaline cells are not so much flattened and in the outer layers of the stroma, especially the outermost, the cell walls are thickened. Beyond this, there is a distinct yellowish brown zone of crushed wood, the cell walls corroded by extra-matrical enzymes; this zone contains numerous hyphae arising from the stroma. No part of the wall arises from the sexual apparatus; it is all stromatic.

The neck of the stromatic perithecium is already partially formed before any asci appear (Fig. 14) and is probably little, if any, taller at this stage than is shown in this figure. It is, at first, a short solid pseudoparenchymatous structure continuous with the wall layers of the stroma. As it grows upwards through the substrate, inwardly growing, curved, hyaline hyphae (which overlap in the centre) form a solid hyaline core within the firm, dark brown cylinder which is obviously an upward extension of the wall of the venter of the ascocarp. There is also some proliferation of the wall layers on the outside of the neck into the cavities of the wood elements. This is much more marked when the fungus is growing on Douglas fir than when it is on alder (Figs. 15, 18).

The central part of the stroma now consists of a basal cushion of small pseudoparenchymatous cells filled with numerous minute oil drops, and a ragged remnant of the same tissue hangs from the ceiling of the central cavity (Fig. 15). Between them stretch the interthelial stroma elements or pseudoparaphyses, a few still complete from top to bottom, but most torn apart at some point along their length by the expansion of the perithecial cavity.

Pushing up between the pseudoparaphyses are asci at all stages of development. These have been formed on the ascogenous hyphae arising from one or more of the sexual cells. The asci are formed on croziers as in *Ceriosporopsis halima*. The group of asci shown in Fig. 16 has been formed by successive lateral proliferations of the crozier.

The asci are asymmetrically clavate and have a uniform, 2-layered wall which is not very thick. There is no pore but there appears to be a pad of clear homogeneous shining cytoplasm at the apex in unstained preparations (Figs. 16, 17). The young asci contain numerous oil drops.

There are usually eight spores in each ascus, but very occasionally seven have been seen. When first cut out from the cytoplasm they are almost cylindrical in form with a clear area at each end where the appendage will be formed (Fig. 16). They gradually become more ellipsoid with the widest part at the centre (Figs. 17, 19). At maturity (Figs. 16, 20-23),

after the single septum has been formed across the middle of the spore, they are very slightly constricted at the septum. The oil drops, which are small and numerous at first, coalesce to form a single large globule in each cell which moves towards the septum. The wall of the spore is 2-layered, the outer clear and shining. The appendages, one at each end, are folded in and curled up against one side of the spore and the whole spore is enclosed in a compact ovoid mass of mucilage formed from the epiplasm (Figs. 16, 17, 19 and 20), as in *C. halima*.

Asci, containing bi-celled ascospores, vary greatly in length. When fully mature they become shorter and greatly expanded laterally. The outer layer of the wall breaks and both the outer and inner layers become mucilaginous as in *C. halima*, thus liberating the cluster of eight ascospores within the perithecial cavity (Fig. 18).

At a stage similar to that in Fig. 15, the hymenial layer may be flat or even concave within the perithecial cavity when seen in a very thick section. When the central region is dissected out from the wall, in sea water, its tissues tear apart in the region which was below the neck and the whole structure immediately turns inside out so that the hymenial layer becomes convex. This indicates a point of weakness below the neck and also suggests the presence of unequal stresses which may well be due to a high turgor pressure resulting from the production of soluble carbohydrates from the oil reserves in the subhymenium.

Later, when most of the asci have liberated their ascospores within the perithecial cavity, all the remaining tissues of the centrum become greatly swollen and then finally disorganized and mucilaginous.

An open passage through the neck is formed by the division, stretching and dissolution of the cells of the wall below the neck (very much as described by Chesters (1938) for *Melanomma pulvis-pyrus*), and by the separation and eventual gelatinization of the hyaline hyphae which form the central core.

A mature perithecium is simply the hollow flask-shaped stromatic wall containing numerous ascospores and mucilage. The ostiole of the one depicted in Fig. 18 has not reached the surface of the wood in which it is immersed. It is not known whether it would continue to grow at the apex or whether the spores, which are already oozing up the neck, must wait for their liberation until the wood has been worn down further.

The appendages of the spores cannot be fully extended as in Figs. 22, 23, until the mucilage surrounding them has expanded and dissolved away. This does not occur immediately if spores freshly liberated from the ascus are mounted in sea water. A certain 'maturation' period, of the order of several days, is necessary. The appendages, like those of *Ceriosporopsis halima*, are both sticky and elastic and readily adhere to any solid object with which they come in contact.

Germination of spores and cultures

The spores germinate readily on sea-water agar within about 48 hr. at room temperature. Most have lost their appendages by this time. A germ tube arises from one or both cells of the spore, frequently terminally,

sometimes subterminally or laterally. The oil reserve in the spore diminishes as the hyphae grow.

The mycelium grows slowly on 0.5 % malt agar made up with sea water. On this medium it forms a greyish cushion surrounded by a dark ring with a colourless submerged margin. It makes hardly any growth on potato agar made with tap water. It has not yet formed any reproductive bodies.

DISCUSSION

That *Ceriosporopsis cambrensis* sp.nov. is distinct from *C. halima* Linder is shown by the discontinuous range in size of their perithecia and spores (Table 2). When seen growing together on the same substrate differences in the colour and distribution of the perithecia are also readily apparent to the naked eye. *C. halima* is the darker.

Table 2. *Dimensions of perithecia, asci and ascospores in Ceriosporopsis halima Linder and C. cambrensis sp.nov.*

	(Measurements in μ .)	
	<i>C. halima</i> *	<i>C. cambrensis</i>
Perithecium	175-400 $\times$ 90-300	577-862 $\times$ 267-548
Length of neck	—	Up to 880
Ascus	—	110-130 $\times$ 24-26
Ascospores	22.5-26-(35) $\times$ 8-10	29-31.5 $\times$ 10.5-14.5†

* From Barghoorn & Linder (1944).

† Measurements of fresh spores in sea water. In herbarium material 3½ years old, spores have shrunk 4 μ in length but less than 1 μ in breadth at septum.

It is worthy of note that there were evidently some spores as much as 35 μ long in the American material of *C. halima* which were not found in the British collections (Tables 1 and 2). Possibly these were giant spores in an abnormal ascus with fewer than eight ascospores.

Linder suggested (Barghoorn & Linder, 1944) that the genus *Ceriosporopsis* may be related to *Bombardia* or to the Sordariaceae. The development of the ascocarp, however, shows that in *Ceriosporopsis halima* and *C. cambrensis* the perithecial wall is derived from the stroma and is not built from vegetative hyphae arising from the ascogonial hypha, nor are true paraphyses, which have a similar origin, present. In other words, the development indicates a relationship, not with the Sphaeriales, but with the Pseudosphaeriales, using these names in the restricted sense recently defined by Miller (1949). The lysigenous opening of the neck or ostiole is also significant, and the structure of the ascus with its uniformly thickened wall and the absence of an apical pore further support this view.

The term 'perithecium', which has been used in this paper in its wider sense of a covering round the asci, requires reconsideration. A term which can be applied to such morphologically different structures as the perithecia in the Sphaeriales and Pseudosphaeriales has lost much of its value and may even be misleading. The use of 'pseudothecium' (e.g. by

Gäumann, 1952) for the stromatic type of ascocarp is an attempt to solve this problem. Although 'theca' (which simply means 'a case') was originally used for the ascus by Hedwig in 1787, the shift in its meaning from ascus to ascocarp has already occurred in such a term as 'cleistothecium'. Possibly this last change has arisen through a confusion between the original and correct meaning of perithecium as something round (preposition) the *asci* and a round (adjective) *case*.

As regards the generic differences between *Ceriosporopsis* and *Ceriospora*, namely, the irregular form of the collapsing perithecia, the deliquescent asci and the deliquescent or dehiscent appendages of the spores, the last two are supported by the present investigation. Although there is some suggestion of a median break in the outer wall of the ascus in the two species which have been described which is reminiscent of that seen by Ingold (1951) in *C. caudae-suis*, the gelatinization of the walls soon obscures this in *Ceriosporopsis*. The structure of the ascus is significantly different from that of *Ceriospora caudae-suis* but much resembles that of *C. duryi* Niessl figured in Rabenhorst (1887).

The irregular shape of the collapsing perithecia has been shown to depend on the substratum in *Ceriosporopsis halima* and it is not a very conspicuous feature in the material examined, so far, of *C. cambrensis*. It is a character which is probably linked with the ability of these fungi to utilize some chemical constituents of the wood and especially of the cell wall more readily than others. Observation of the position occupied by the mycelium in the xylem elements and in the medullary rays, the zone of crushed and partly digested wood surrounding the perithecia, and the experimental work of Barghoorn (Barghoorn & Linder, 1944) on *C. halima*, taken together, suggest that these fungi can break down the primary wall or middle lamella more readily than the secondary or tertiary layers. The irregular form of the perithecium can be a helpful feature in the recognition of the genus if it is used with caution and understanding of its possibly physiological basis.

Finally, Linder's definition of the genus *Ceriosporopsis* needs to be slightly emended to include the following observations recorded in this paper: (1) There is a long perithecial neck which is gradually worn down as the wood in which it is immersed is eroded. (2) The colour of the perithecial wall is not necessarily dark. (3) The asci are 8-spored.

My very grateful thanks are due to Mr E. W. Mason for the Latin diagnosis, to Dr E. E. Watkin for identifying the Crustacea and to Prof. C. G. C. Chesters and Mr E. H. Chater for advice on various other aspects of the work.

REFERENCES

- BARGHOORN, E. S. & LINDER, D. H. (1944). Marine fungi; their taxonomy and biology. *Farlowia*, **1**, 395-467.
 CHESTERS, C. G. C. (1938). Studies in British Pyrenomycetes. *Trans. Brit. mycol. Soc.* **22**, 116-150.
 GÄUMANN, E. A. (1952). *The Fungi*. Translated from the German and arranged as an American text by F. L. Wynd. New York and London: Hafner.
 INGOLD, C. T. (1951). Aquatic ascomycetes: *Ceriospora caudae-suis* n.sp. and *Ophiobolus typhae*. *Trans. Brit. mycol. Soc.* **34**, 210-215.

- MILLER, J. H. (1928). Biologic studies in the Sphaeriales, I. *Mycologia*, **20**, 187-213.
 MILLER, J. H. (1949). A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. *Mycologia*, **41**, 99-127.
 RABENHORST, L. (1887). *Kryptogamen-Flora, II. Ascomyceten*. Leipzig.
 WILSON, I. M. (1951). Notes on some marine fungi. *Trans. Brit. mycol. Soc.* **34**, 540-543.

(Accepted for publication 5 November 1953)

STUDIES ON A CANKER DISEASE OF CYPRESSES IN EAST AFRICA, CAUSED BY *MONOCHAETIA UNICORNIS* (COOKE & ELLIS) SACC.

II. VARIATION IN THE MORPHOLOGY AND PHYSIOLOGY OF THE PATHOGEN

By D. RUDD JONES

East African Agriculture and Forestry Research Organization, Kikuyu, Kenya,
and National Research Council, Prairie Regional Laboratory,
Saskatoon, Saskatchewan, Canada*

(With Plates 10 and 11, and 5 Text-figures)

Monochaetia unicornis occurs naturally in three strains. Strain A is virulent and fast-growing in culture, but produces few acervuli. Strain B is less virulent and slower growing, but produces many acervuli. Strain D is parasitic only on *Juniperus procera*, grows at a similar rate to B and rarely produces acervuli in culture. It is strongly antagonistic to A and B. Strain C comprises a range of biotypes which have arisen in culture and which are saprophytic and slow-growing.

Comparisons between the size and seta-length of conidia from fifty collections showed that seta-length was the only dependable morphological character for separating A and B, the setae being significantly longer in the latter. Strain D conidia were wider and darker in colour than those of A and B.

Optimal temperature for growth of strains A and B was 25° C. and for D, 20° C. Growth on various media showed strain A to have the most 'parasitic' type of metabolism, with B intermediate, and D having a 'saprophytic' type. Mycelial growth of A and B was optimal at 97.5% relative humidity, whilst a saturated atmosphere was more favourable for germination of their conidia. Strain D had a similar growth rate over a wide range of relative humidity but conidia germinated best at 97.5% R.H. Strains A and B grew best on an agar medium to which 10–20% sucrose had been added, whilst D grew best on 40% sucrose. Strains B and D could tolerate higher osmotic pressures in liquid culture than A and conidia of D germinated best at 0.4 atm.

It is suggested that the distribution of strains B and D is limited by climate, B being able to compete with A only on dry sites. Strains A and B are considered to be biotypes of one species whereas D is a separate species or variety.

INTRODUCTION

In a previous paper (Jones, 1953) reference was made to the existence of four 'strains' or biotypes of *Monochaetia unicornis* (Cooke & Ellis) Sacc. Field observations and cultural studies showed that these strains could be distinguished by certain characteristics to give tentative descriptions of the strains as follows:

Strain A: a virulent pathogen producing extensive cankers in inoculated trees. In culture on complete agar media, it is fast-growing but forms few acervuli. The conidia are long, narrow, and nearly hyaline, with short setae. They retain their viability for longer periods than those of strain B.

* Now at Imperial Chemical Industries Ltd., Butterwick Research Laboratories, The Frythe, Welwyn, Hertfordshire.

Strain B: less virulent than A. In culture it is slower growing than A but forms many acervuli. The conidia are generally shorter than those of A, but wider and darker in colour, and they have longer setae.

Strain C: comprises a range of isolates which have arisen in culture and which are non-pathogenic, slower-growing than B, and sterile.

Strain D: non-pathogenic on all *Cupressus* species tested but causes typical, though limited, cankers on *Juniperus procera* from which it was originally isolated. At temperatures above 25° C it grows slowly in culture, but at 20° C the growth rate is comparable with, or higher than, that of strain B.

The present paper is concerned with a more detailed examination of the differential characteristics to determine their value in the morphological and physiological separation of the strains, and with the significance of strain differences in the etiology of cypress canker in East Africa.

SOURCES OF ISOLATES

Collections of the fungus were made from *Cupressus* species in all the plantation areas of Kenya, Tanganyika and Uganda where the disease is active. In an effort to obtain isolates from as wide a range of substrates as possible, a search was made in naturally occurring *Juniperus procera* and *Podocarpus gracilior*, and in plantations of these species, the only indigenous conifers.

Single spore cultures were established by streaking suspensions of conidia, in sterile water, on potato dextrose agar (P.D.A.) plates. Separation of conidia was checked under the microscope and transfers were made from single colonies after 3–4 days at room temperature (20° C.). Table 1 gives the sources of the collections of the fungus which have been studied in culture, together with six collections isolated by Dr R. M. Nattrass.

APPEARANCE OF THE FUNGUS IN CULTURE

Cultures grew well on Difco P.D.A. (potato dextrose agar) with 0.5 % yeast extract added, and on this medium the four strains could readily be distinguished:

Strain A. Colonies floccose, woolly, with ragged margins (Pl. 1, fig. 1). Hyphae in normal colony thin at margin (1–3 μ), freely branching, cross-septa frequent only under unfavourable conditions of growth; hyphae in staling colony up to 6 μ diameter, with swellings at the nodes. Chlamydospores thick-walled, up to 8 $\times$ 10 μ , or spherical 6–8 μ . Colour with normal growth, white or light buff at periphery shading to Ivory Yellow or Citrine Drab at centre (Ridgway, 1912); under less favourable conditions of growth, Seashell Pink at periphery, Deep Olive at centre.

Strain B. Colonies similar in appearance to A but with less aerial growth and ragged, almost rhizoidal, margins (Pl. 1, fig. 2). Hyphae thicker than in A (2–5 μ) with more frequent branching to form denser colonies. Chlamydospores formed as in strain A. Colour Seashell Pink on the periphery shading to Salmon Buff at the centre; under unfavourable conditions of growth, colour deeper (Pompeian Red) and pigmentation apparent in medium.

Table 1. *Sources of collections of strains of Monochaetia unicornis on Cupressus macrocarpa, C. lusitanica, C. forbesii and Juniperus procera*

Coll. no.	Strain	Host and year of planting	Spore	Locality	Isolation date
RM 1*	B	<i>C. mac.</i> 1927	Conidia	Bahati K	Dec. 1949
RM 2	A	<i>C. lus.</i> 1946	Conidia	Kigumo K	Feb. 1950
RM 3	A	<i>C. lus.</i> 1946	Conidia	Kigumo K	Feb. 1950
RM 4	A	<i>C. lus.</i> 1948	Conidia	Kiandongoro K	Feb. 1950
RM 5	B	<i>C. lus.</i> 1947	Conidia	Nanyuki K	Feb. 1950
RM 6	A	<i>C. mac.</i> 1945	Conidia	S. Kinangop K	Mar. 1950
RM 7	A	<i>C. mac.</i> 1934	Conidia	Kaptagat K	Mar. 1950
RM 8	A	<i>C. mac.</i> 1947	Conidia	Kerita K	Mar. 1950
RM 9	A	<i>C. mac.</i> 1934	Conidia	Uplands K	Mar. 1950
RM 10	A	<i>C. mac.</i> 1936	Conidia	Kaptagat K	Mar. 1950
RM 11	A	<i>C. mac.</i> 1943	Conidia	Kipkabus K	Mar. 1950
RM 12	A	<i>C. mac.</i> 1943	Conidia	Kaptagat K	Mar. 1950
RM 13	A	<i>C. mac.</i> 1944	Conidia	Kipkabus K	Mar. 1950
RM 14	A	<i>C. mac.</i> 1945	Conidia	Kerita K	Apr. 1950
RM 15	A	<i>C. mac.</i> 1946	Conidia	Kerita K	Apr. 1950
RM 16	A	<i>C. mac.</i> 1927	Conidia	Uplands K	Apr. 1950
RM 17	A	<i>C. lus.</i> 1946	Conidia	Hombe K	Apr. 1950
RM 18	A	<i>C. lus.</i> 1944	Conidia	Hombe K	Apr. 1950
RM 19	A	<i>C. mac.</i> 1932	Conidia	Hombe K	Apr. 1950
RM 20	A	<i>C. mac.</i> 1946	Conidia	S. Kinangop K	Apr. 1950
RM 21	A	<i>C. lus.</i> 1946	Conidia	Chehe K	May 1950
RM 22	A	<i>C. mac.</i> 1945	Conidia	Uplands K	May 1950
RM 23*	B	<i>C. forb.</i> 1942	Conidia	Elburgon K	May 1950
RM 24	A	<i>C. mac.</i> 1948	Conidia	S. Kinangop K	May 1950
RM 25	A	<i>C. lus.</i> 1948	Conidia	S. Kinangop K	May 1950
RM 26	A	<i>C. mac.</i> 1939	Conidia	Sabatia K	May 1950
RM 27*	A	<i>C. mac.</i> 1936	Conidia	Elgeyo K	May 1950
RM 28	A	<i>C. mac.</i> ?	Conidia	Machakos K	Aug. 1950
RM 29	A	<i>C. mac.</i> ?	Conidia	Machakos K	Aug. 1950
RM 30	A	<i>C. mac.</i> 1947	Conidia	Shume-Magamba T	Oct. 1950
RM 31	A	<i>C. lus.</i> 1942	Conidia	Shume-Magamba T	Oct. 1950
RM 32	A	<i>C. lus.</i> Seedling natural regeneration	Conidia	Shume-Magamba T	Oct. 1950
RM 33	A	<i>C. lus.</i> ?	Conidia	Tukuyu T	Nov. 1950
RM 34	A	<i>C. lus.</i> 1940	Ascospores	Kabage K	Feb. 1951
RM 35*	B	<i>C. mac.</i> 1920	Conidia	Kabale U	Mar. 1951
RM 36	A	<i>C. mac.</i> 1937	Conidia	Meri-Thego K	Aug. 1951
RM 37	A	<i>C. mac.</i> 1937	Ascospores	Meri-Thego K	Aug. 1951
RM 38	B	<i>C. mac.</i> 1929	Conidia	Bahati K	Aug. 1951
RM 39	B	<i>C. mac.</i> ?	Conidia	Bahati K	Aug. 1951
RM 40	A	<i>C. mac.</i> 1942	Conidia	Bahati K	Aug. 1951
RM 41	B	<i>C. mac.</i> 1942	Conidia	Bahati K	Aug. 1951
RM 42	A	<i>C. mac.</i> 1946	Conidia	Bahati K	Aug. 1951
RM 43	B	<i>C. lus.</i> 1946	Conidia	Nanyuki K	Sep. 1951
RM 44*	D	<i>J. proc.</i> ?	Conidia	Nanyuki K	Sep. 1951
Natural regeneration					
RM 45	A	<i>J. proc.</i> ?	Conidia	Kabage K	Oct. 1951
RM 46	B	<i>C. mac.</i> ?	Conidia	Ndaragwe K	Apr. 1952
NM 24	A	? ?	Conidia	Kerita K	?
NM 229	C	<i>C. mac.</i> 1944	Conidia	Nanyuki K	Jan. 1949
NM 232	B	<i>C. mac.</i> 1933	Conidia	Londiani K	Mar. 1949
NM 233	C	<i>C. mac.</i> 1933	Conidia	Londiani K	Mar. 1949
NM 1313	A	<i>C. mac.</i> ?	Ascospores	? K	Jan. 1950
NM 1523	A	<i>C. mac.</i> 1945	Ascospores	Kerita K	?

RM=collections made by the writer; NM=collections made by Dr R. M. Nattrass; K=Kenya; T=Tanganyika; U=Uganda.

* Cultures deposited at the Commonwealth Mycological Institute are marked with an asterisk.

Strain C. Colonies small, dense, with discrete margins. Hyphae branch frequently, vary considerably in size ($1-6\ \mu$) and there are many cross-septa. A large part of the growth is submerged. Chlamydospores as in strains A and B. Colour, in isolates derived from strain A, uniform white; in isolates derived from B, often deeply pigmented to Pompeian Red.

Strain D. Colonies dense, with less aerial growth than strain A, and ragged margins (Pl. 1, fig. 3). Under less favourable conditions of growth margins discrete, colonies rapidly stale medium. Hyphae similar in size and appearance to strain A, chlamydospores formed in the same way. Colour, uniform white developing to Warm Buff with time. An orange pigment diffuses into the medium and appears to be concentrated in amorphous deposits, up to $15\ \mu$ in diameter, closely associated with hyphae.

Antagonism in culture

Cultures of collections of strain A were found to be completely compatible when grown together on P.D.A. plates (Pl. 1, fig. 4). The same was true for collections of strain B, including the rather atypical collection RM 35. Collections of the two strains were also mutually compatible, although a line of demarcation between colonies was obvious in heavier aerial growth in the approaching margins (Pl. 1, fig. 5). There was a marked antagonism and inhibiting effect on the growth of both A and B strains by cultures of strain D (Pl. 1, fig. 6). The hyphae in the approaching margins of two colonies were found to be much branched, shortened, and with many cross-septa (Pl. 2, figs. 1, 2). These effects were always more pronounced in the hyphae of strains A and B than in those of D. In one instance where strain D (RM 44) was grown with strain B (RM 1), acervuli were formed along the approaching margin of the strain B collection.

Certain aerobic spore-forming bacteria, occurring on plates as contaminants, inhibited mycelial growth and acervulus production in both A and B strains. Two species of *Aspergillus* stimulated mycelial growth in both strains but acervulus production was delayed. Strain D inhibited the growth of a number of fungi.

PRODUCTION OF ACERVULI IN CULTURE

In natural infections and inoculated trees acervuli erupt through the bark 4-6 weeks after infection. They are formed more rapidly following inoculations with strain A than with strains B and D. In culture, acervuli have been produced on sterilized *Cupressus* and *Juniperus* twigs and on various agar media, including P.D.A. plus 0.5 % yeast extract, and 2 % malt agar. Table 2 gives details of the size and time of production of acervuli in culture. On sterilized twigs the acervuli appear similar to those on natural cankers although they are generally more superficial, but this may be due to the small physical resistance of the phellogen (after autoclaving) to the eruption of acervuli. With strain B collections, the acervuli produced in twig culture are much larger than those on natural cankers. On agar media, the stomata of all strains are very limited.

The optimal temperature for acervulus production on agar media varied for the three strains. Acervuli were first formed at temperatures

other than those optimal for vegetative growth. Thus, with collections of strains A and B acervuli first appeared at incubation temperatures of 20° or 30° C., whereas the optimal temperature for vegetative growth is near 25° C. Similarly, with strain D acervuli first appeared at 15° C., although the optimal temperature for vegetative growth is near 20° C. With time, however, more acervuli were formed always at the temperature optimal for vegetative growth, but production did not commence until vegetative growth was restricted by the edges of the plate or by staling of the medium. Acervuli were produced in abundance by all collections of strain B on

Table 2. *Production of acervuli in culture by Monochaetia unicornis*

Coll. no.	Strain	Medium and temp.	Time for appearance of ripe acervuli (days)	Mean diameter and range (mm.)	Depth of stroma (μ)
RM 40	A	Twig (20° C.)	12	0.22-0.38-0.66	80-300
RM 43	B	Twig (20° C.)	5-8	0.42-0.93-1.42	80-300
RM 44	D	Twig (20° C.)	24	0.19-0.34-0.66	80-300
RM 40	A	P.D.A.* (20° C.)	14	—	—
RM 43	B	P.D.A.* (20° C.)	7	—	—
RM 44	D	P.D.A.* (15° C.)	24-30	—	—

* Plus 0.5% yeast extract.

agar media but only infrequently by collections of strain A and rarely by strain D. Collections of strain A varied in their ability to form acervuli. RM 6 and RM 40 would normally produce acervuli in 2 weeks and were used for all experimental work, but other collections either completely failed to produce acervuli or did so rarely. In all the early studies, strain D (RM 44) failed to produce acervuli but one subculture on P.D.A. kept at 5° C. for 6 months showed acervulus initials, and these produced ripe conidia after incubation at 20° C. Subsequently, two or three acervuli were formed on one plate out of five at 15° C., but acervulus production was never improved in this strain.

Conidia produced on agar media were normal in appearance, although the percentage germination was frequently lower than in field collections. If the medium was allowed to dry out during incubation, one or more of the medial cells of a conidium often developed into thick-walled chlamydospores.

With collections of strain B, and particularly with RM 35, the production of acervuli in agar culture was often similar to that found in canker infections (Pl. 2, fig. 3). Following a period of vegetative growth a ring of acervuli was formed and this was followed by a further period of vegetative growth and the formation of a second ring of acervuli.

Apparently there is a mycelium 'age' factor, the expression of which must reach a certain threshold before acervuli initials can be formed. The threshold value is different for each strain. All attempts to simulate the age factor chemically have been unsuccessful. Culture filtrates of strain B collections were added to agar cultures of strains A and D, but failed to stimulate more rapid production of acervuli. Alterations in the carbon source, the nitrogen source and the C/N ratio did not affect the time of

acervulus production by any strain, although addition of 0.5 % yeast extract to P.D.A. did improve the numbers of acervuli formed. With collections of strain B the age factor may be expressed, in part, by any check on mycelial growth such as drying-out of the medium or antagonistic effects of other organisms. As reported above, incubation at temperatures unfavourable for vegetative growth stimulates the more rapid formation of acervuli in all three strains.

As with agar cultures, a delay in the production of acervuli is experienced with twig cultures, although acervuli are formed in much larger numbers by all three strains.

All attempts to produce perithecia in culture, with the exception of perithecium production in a twig culture previously reported (Jones, 1953), have been unsuccessful.

APPEARANCE AND SIZE OF CONIDIA

Examination of conidia from various sources revealed that they differed in size and colour, not only between the three strains but also between collections of the same strain from different sources.

The conidium is six-celled, the four medial cells being coloured and the end cells hyaline. When the conidium is ripe it either breaks away from the pedicel to which it is attached, or a portion of the pedicel may remain

Table 3. *Conidial size of collections of Monochaetia unicornis*

Strain	No. of samples*	Source of conidia	Mean length of conidium	Mean width of conidium	Mean length of seta
A	4	Natural canker	13.18	3.79	3.55
	1	Natural canker	13.30	3.60	5.40
		<i>J. procera</i>			
	8	Inoculated <i>C. macrocarpa</i>	14.45	3.63	3.12
	30	Inoculated <i>C. lusitanica</i>	14.55	3.72	3.07
	7	Twig culture	16.46	3.78	4.25
	2	P.D.A.	16.57	3.41	3.85
	2	2 % malt agar	14.42	3.94	3.10
B	5	Natural canker	14.20	4.49	4.94
	3	Inoculated <i>C. macrocarpa</i>	15.05	4.47	6.90
	3	Inoculated <i>C. lusitanica</i>	15.95	4.44	4.45
	2	Twig culture	15.15	4.49	8.05
	4	P.D.A.	13.52	4.11	8.57
	3	2 % malt agar	13.40	4.31	6.71
D	1	Natural canker	17.00	5.83	3.40
		<i>J. procera</i>			
	2	Twig culture	17.42	5.20	4.97
	1	P.D.A.	15.15	4.22	3.70

Units of measurement are micrometer eyepiece readings; 1 unit = 1.66 μ .

* Twenty conidia in each sample.

attached to an end cell. The other end cell bears a single seta, or occasionally two in collections of strain B. Conidia were collected from natural cankers, and from inoculated trees, and were produced in culture. Conidia were mounted in glycerine jelly and the length, width, and seta length of twenty conidia from each sample of a collection were measured. The summarized means are shown in Table 3.

Each set of data from twenty individuals was analysed to see if there was any strong relationship between length and seta length. Of seventy sets of data examined, twenty-two showed a negative correlation and forty-eight a positive correlation. However, only seven of these correlations were significant ($P = \text{or} < 0.05$), six positive and one negative. In spite of the small number of sets showing a significant relation, there was evidence of a general positive association within each A set, but this is not helpful in separating the strains.

Inspection of the data on conidium width showed that the conidia of strain D were wider than those of A and B except on P.D.A. Conidia of strain B were wider than those of A on most substrates but occasionally collections of strain A produced wide spores and there was no distinction between the two strains on this basis.

Several independent analyses of variance were performed on the data for length of conidium and length of seta to determine the value of these characters in separating the strains. In eleven analyses involving A and B strains, four showed A as having significantly longer conidia than B, and one B longer than A. There were two analyses with opposite results on different substrates, and four with no significant difference between strains. With seta length, ten showed B as having longer setae than A, and one showed no significant difference in inoculated *Cupressus macrocarpa* but longer setae in B on P.D.A. In two analyses involving A and D strains, D had longer conidia from one source only (*Juniperus procera*) and A had significantly longer setae on the same substrate. B conidia were significantly shorter than D and the setae significantly longer. With samples of conidia from different substrate sources, strain A generally had longer conidia than B in culture on 2 % malt agar, P.D.A. and twig, but there were no consistent significant differences between the two strains in conidia from natural cankers or inoculated trees. Generally, too, conidia of all strains were longer from twig cultures than from natural cankers and inoculated trees, and they in turn were longer than conidia from cultures on 2 % malt agar or P.D.A. Conidia produced in culture frequently had longer setae than those from natural cankers or inoculated trees but there were many exceptions, especially in the twig cultures.

For one analysis, conidia were collected from different crops of acervuli on the same trees with a year's interval between collections. There were no significant differences in conidial size between the two times of collection.

Colour differences in the conidia of the three strains tended to be accentuated in culture. The conidia of strain A were thin-walled and hyaline, those of strains B and D were thinner-walled and lighter in colour than in natural cankers but they still contrasted strongly with strain A (Pl. 2, figs. 4-6). In natural cankers and inoculated trees, the differences were not so obvious, and it was often difficult to distinguish between A and B collections although the very dark coloration of strain D conidia was usually obvious.

Analysis of the conidia size data has shown that seta length is the only satisfactory criterion for separating A and B strains morphologically, and strain D is best distinguished from B by the greater width, darker colour, and shorter setae.

GROWTH OF THE FUNGUS IN INOCULATED TREES

There are no known differential hosts for the strains of *Monochaetia unicornis*. Collections of strains A and B produced canker lesions in a number of species (Jones, 1954). Strain D produced cankers in *Juniperus procera* only, although it will persist for short periods in other host species, but presumably only as a saprophyte.

There are distinct differences in virulence between collections of A and B strains and studies were made to assess these differences. The only satisfactory method of recording the pathogenicity of a collection has been to measure the longitudinal extension of cankers produced by standardized methods.

Longitudinal extension of cankers

1 cm. vertical slits, penetrating to the secondary wood, were made into the stems of 2-year-old *Cupressus lusitanica* trees at the same height from the ground. 5 mm. disks of agar bearing mycelium were cut from the periphery of 10-day cultures incubated at 20° C. on P.D.A., and inserted into the slits, which were afterwards sealed with 'Selotape'. After 3 weeks, measurements of the longitudinal extension of cankers were made and then repeated again at 2-week intervals for 1 year.

Separate plots of ten trees were inoculated with each collection. The means of ten readings of canker length, 7 weeks after inoculation, are recorded in Table 4. Differences between collections of the two strains were found to be highly significant ($P < 0.001$) with collections of strain A always more virulent than those of strain B. Differences between collections of strain B were not significant but there was a highly significant difference ($P < 0.001$) between collections of strain A. This was surprising in view of the results obtained in cultural studies (see p. 297) where the collections were remarkably consistent and greater variation was apparent in collections of strain B. However, the host population used for the inoculations was known to be heterogeneous. Furthermore, with the small number of trees in each sample, and differences in the host reaction of individual trees, these results might have been expected, and do not necessarily indicate profound differences in virulence between collections of strain A. The results with strain B were more uniform because of the small number of collections used, and the limited extension of cankers.

Occlusion of cankers and recovery were more successful following inoculations with strain B than with strain A. One year after inoculation 65.0 % of the cankers produced by strain A collections were still active as compared with 30.0 % for strain B collections.

Although both A and B strains had been found in the same plantation, they were never found on the same tree (Jones, 1953). When collections of both strains were inoculated at points close together on the same tree, the canker caused by the strain A collection would overrun the canker caused by the strain B collection and isolations from the bark or from acervuli would yield strain A only. Where the two points of inoculation were further apart, the strain B canker would develop normally and acervuli would be formed from which strain B could be re-isolated. No

tests of the antagonism of strain D to strains A and B in inoculated *Juniperus* have been made as yet.

Table 4. *Longitudinal extension of cankers in inoculated trees and growth of Monochaetia unicornis on potato dextrose agar*

Coll. no.	Strain	Mean length of canker after 7 weeks (mm.)	Corrected mean diameter of colony (mm.) after 7 days at 26.5° C. on
			P.D.A.
RM 1	B	16.1	16.54
RM 2	A	40.6	25.90
RM 3	A	44.2	27.15
RM 4	A	45.4	—
RM 5	B	25.5	14.10
RM 6	A	42.9	25.29
RM 7	A	40.2	23.87
RM 8	A	34.1	24.65
RM 9	A	44.4	26.16
RM 10	A	42.2	24.67
RM 11	A	34.0	25.87
RM 12	A	38.4	22.70
RM 13	A	37.9	28.55
RM 14	A	50.3	26.64
RM 15	A	49.8	26.20
RM 16	A	39.5	25.26
RM 17	A	36.9	25.96
RM 18	A	28.6	26.25
RM 19	A	40.8	26.00
RM 20	A	33.5	27.37
RM 21	A	36.5	25.60
RM 22	A	54.2	25.12
RM 23	B	21.3	16.37
RM 24	A	48.8	25.80
RM 25	A	32.9	24.13
RM 26	A	36.8	26.75
RM 27	A	31.4	26.45
RM 28	A	25.5	24.56
RM 29	A	33.5	27.00
RM 30	A	33.1	24.60
RM 31	A	35.1	28.10
RM 32	A	28.2	23.75
RM 33	A	35.9	27.30
RM 34	A	—	26.48
RM 35	B	—	17.15
RM 38	B	—	16.90
RM 39	B	—	14.70
RM 40	A	—	26.90
RM 41	B	—	13.70
RM 42	A	—	25.30
RM 43	B	—	22.40
RM 44	D	—	12.81
RM 45	A	—	23.20
RM 46	B	—	15.17
NM 24	A	38.8	—
NM 232	B	—	15.85
NM 1313	A	33.4	—
NM 1523	A	33.6	—

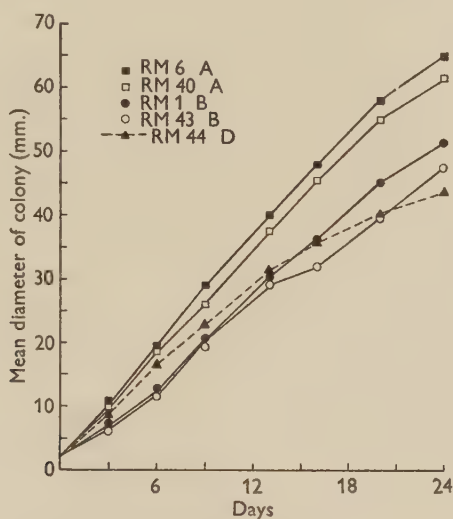
GROWTH OF THE FUNGUS IN ARTIFICIAL CULTURE

Monochaetia unicornis grows slowly in culture, and in the early part of the investigation there were no facilities for growth in liquid culture and the determination of dry weights of mycelium as a measure of growth. There-

ore, the linear extension of colonies on agar media was used as a measure of growth and was subsequently compared with dry weights of mycelium (Table 5). This comparison showed that strains A and B gave comparable dry-weight : mean-diameter ratios at optimal temperatures for mycelial

Table 5. Comparison of dry weights of mycelium with linear measurements of colonies in the growth of *Monochaetia unicornis* on P.D.A. + 0.5 % yeast extract

Coll. no.	Strain	Incubation temp. ° C. for 28 days	Dry wt. of mycelium (mg.)	Mean diameter of colony (mm.)	Ratio Dry wt./mean diam.
RM 5	B	30.0	474.7	26.30	18.049
RM 5	B	22.5	628.3	63.95	9.825
RM 35	B	22.5	760.7	66.75	11.396
RM 40	A	30.0	696.9	37.65	18.510
RM 44	D	22.5	561.1	39.80	14.098



Text-fig. 1. Growth of strains of *M. unicornis* on P.D.A. + 0.5 % yeast extract.

growth, although collections of strain A tended to produce heavier colonies because of more profuse aerial growth.* At higher and at lower temperatures, the strains were again comparable but the colonies of both were more dense. Strain D formed heavier colonies than A and B at all temperatures, although this is due, in part, to the more rapid staling of the medium by this strain. Text-fig. 1 illustrates the growth rates of two collections of strain A, two of strain B and the one collection of strain D. Inspection of the growth-rate curves confirmed that, with mycelial inoculations from 19-day P.D.A. plates kept at 20° C. (mean of five plates for each point) readings taken after 7 days came in the period of maximum growth rate for all three strains. By 7 days, the lag phase for collections of

* RM 35 (Pl. 2, fig. 3) is intermediate since it forms more aerial growth than typical strain B collections.

strain B had been passed and the falling-off in the growth of strain D was not yet discernible.

Preliminary experiments to compare the growth of collections were undertaken in a field laboratory. As it was not possible to repeat conditions of medium and incubation exactly, a collection of strain A and another of B were included in each experiment as control checks. The medium used was P.D.A. (20 ml. per plate) prepared from locally grown potatoes. The inoculum was 2 mm. diameter disks taken from the periphery of 10-day P.D.A. plate cultures kept at 20° C. (this type of inoculum was used in all subsequent growth experiments). Incubation was at 26.5° C. and two diameters at right angles to each other were measured on each colony after 7 days' incubation, to the nearest 0.5 mm. In the earlier experiments, four plates were used for each collection but this was later increased to five. Table 4 gives the means of diameter readings corrected against the control checks. There was a strong positive correlation ($P=0.01-0.001$) between length of canker and colony diameter for thirty-two collections where there were data for both variables.

Analyses of variance were performed on the original data of eight experiments. The variation between plates was used as the error variance, and this was always greater than the variation within plates ($P=0.01$, $P=0.05$ in one run only). The large differences between collections of strain A and collections of strain B and D were apparent throughout. Even if the variation between strains was used to test the significance of the difference between A and B strains, the difference was so great that in no case did the higher variation between strains with its fewer degrees of freedom cause the difference to be statistically non-significant. Within strains there was sometimes considerable variation and inconsistent performance in a control check. RM 13 grew more rapidly ($P<0.001$) than seven other collections of strain A in the same run. In another run RM 5 produced much smaller colonies than the other B collections ($P<0.001$) and RM 27 produced larger colonies than the other A collections in the same run ($P<0.05$). In a third run RM 43 produced much larger colonies than the other B collections ($P<0.001$), although the difference between A and B strains in this run was still highly significant ($P<0.001$). All these collections subsequently grew normally for their strains and unobserved differences in the inocula may have been responsible for the inconsistencies. Apart from these exceptions there was no significant difference between collections of one strain.

Effect of temperature on growth

Difco P.D.A. plus 0.5 % Difco yeast extract was used as the medium and incubation was controlled at 15°, 20°, 25° and 30° C. A high relative humidity was maintained in the incubators by exposing areas of continuously moist filter-paper. Fourteen collections of three strains were used and measurements were made to the nearest 0.5 mm. with a modified Fisher-Lilly antibiotic zone reader.

The results are shown in Table 6 and an analysis of variance performed on the data in Table 7. In the first instance variation between plates was used as the error variance and this indicated that many of the differences

Table 6. *Effect of temperature on the growth of Monochaetia unicornis on P.D.A. + 0.5 % yeast extract*

Coll. no.	Strain	Mean diameter of colony (mm.) after 7 days			
		15° C.	20° C.	25° C.	30° C.
RM 1	B	17.80	18.85	21.62	10.05
RM 5	B	14.70	18.05	21.60	9.85
RM 6	A	19.45	25.00	31.95	15.70
RM 17	A	20.40	24.05	30.95	14.70
RM 21	A	19.50	24.25	30.95	19.00
RM 23	B	12.20	15.50	18.31	10.15
RM 27	A	20.00	24.55	31.15	18.65
RM 35	B	16.85	18.30	22.31	11.10
RM 38	B	13.65	17.65	19.95	11.06
RM 40	A	19.90	24.25	30.60	17.25
RM 41	B	12.85	15.85	17.87	11.00
RM 43	B	14.00	16.05	16.10	11.44
RM 44	D	15.45	21.30	18.50	2.56
RM 45	A	19.50	24.35	31.00	20.90

Table 7. *Analysis of variance applied to growth data of Table 6*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	539	20,503.34	—
Within plates	270	153.62	0.57
Between plates	269	20,349.72	—
Temperatures	3	9,418.40	3139.47
Collections A	5	39.67	7.93
B	6	333.82	55.64
A v. B	1	7,442.20	7442.20
D v. (A + B)	1	579.87	579.87
A × temperature	15	240.42	16.03
B × temperature	18	336.20	18.68
(A v. B) × temperature	3	545.85	181.95
(D v. (A + B)) × temperature	3	835.43	278.48
Error	214	577.87	2.70

were highly significant even between collections of strain A ($P=0.05$). However, it was apparent that the behaviour of the various collections was not consistent over the four incubation temperatures, and therefore it appeared that a more accurate assessment of significance could be obtained by using the mean of the interaction of strain A with temperature and strain B with temperature as the error variance. On this basis, differences between temperatures of incubation, A and B strains, D v. (A + B), A v. B interaction with temperature, and D v. (A + B) interaction with temperature were all found to be highly significant ($P<0.001$). Differences between collections of strain A were not significant but collections of strain B did differ significantly ($P=0.05-0.01$). Surprisingly, the uniformity of the B collections at 30° C. was high, whereas the A collections were more variable. It may be that the population comprises collections with different optimal temperature requirements. Certainly, RM 45 is exceptional in growing better than the other A collections at 30° C., and this cannot be attributed to unobserved inoculum differences since these would be the same at all temperatures.

Variations in collections of strain B have been noted throughout these studies. In one experiment involving three collections of strain B, six

plates out of ninety showed sectoring. Sectoring has been seen very rarely in collections of strains A and D. Further, RM 35, which was collected in Uganda, produced colonies which closely resembled those of strain A and which were generally larger than other collections of strain B at 25° C., although at 15° and 20° C. RM 1 produced the largest colonies.

Effect of pH on growth

P.D.A. plus 0.5 % Difco yeast extract plates were made up at pH 5.0, 6.0 and 7.0 without additional buffering. The growth of five collections in 7 days is shown in Table 8 (each mean determined from five plates). The optimal pH for strain A, as represented by RM 40, was near to 6, whereas optima for strains B and D were nearer pH 5.0. Strain A grows well over a wide range of pH and this is in accordance with results obtained by Ciccarone (1949).

Table 8. *Effect of pH on the growth of Monochaetia unicornis on P.D.A. + 0.5 % yeast extract*

Coll. no.	Strain	Mean diameter of colony (mm.) after 7 days at 20° C.		
		pH 5.0	pH 6.0	pH 7.0
RM 5	B	15.75	13.50	12.50
RM 35	B	20.05	15.65	13.75
RM 40	A	24.95	25.45	24.40
RM 43	B	17.00	11.15	9.75
RM 44	D	20.40	19.40	16.90

S.E. of means = 1.17.

Effect of medium on growth

Comparisons were made of the growth of collections of the three strains on four media. The results are shown in Table 9. Yeast extract added to Czapek-Dox agar improved the growth of all strains, although the improvement was less pronounced in strains B and D than in A. Addition of peptone improved the growth of strains A and B but the improvement was slight with A. The addition of peptone limited the growth of strain D even though the colonies appeared normal and healthy and the growth

Table 9. *The growth of collections of Monochaetia unicornis on culture media*

Coll. no.	Strain	Mean diameter of colony (mm.) after 7 days at 20° C.			
		1*	2*	3*	4*
RM 5	B	6.85	9.35	17.00	15.80
RM 35	B	8.25	14.20	18.75	17.85
RM 40	A	10.37	17.75	18.20	24.20
RM 43	B	5.60	8.30	13.35	14.45
RM 44	D	17.05	23.55	17.25	19.15

S.E. of means = 2.40.

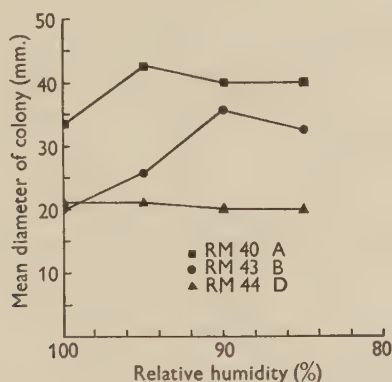
* Media: 1, Czapek Dox; 2, Czapek Dox + 0.5 % Difco yeast extract; 3, Czapek Dox + 0.5 % Difco yeast extract + 0.5 % peptone ('Phytone' produced by Baltimore Biological Laboratory, Baltimore, Maryland); 4, Difco potato dextrose agar + 0.5 % Difco yeast extract (pH adjusted to 5.6 in all media).

as only slightly less than the same strain on P.D.A. It is not clear whether the peptone is toxic to strain D or whether the addition of peptone to the medium lowers the C/N ratio to a level unfavourable for the growth of strain D. If we consider that strain D has a more 'saprophytic' metabolism than strains A and B then the latter explanation would seem reasonable. Collections of strain B showed similar or slightly less growth on P.D.A. plus yeast extract to that on Czapek-Dox agar plus yeast extract plus peptone, but strain A showed much more extensive growth and this might be expected of a strain with a more 'parasitic' metabolism.

An analysis of variance performed on the data of Table 9 showed no significant difference between the growth of collections on different media ($P=0.01-0.001$).

Effect of humidity on growth in agar culture

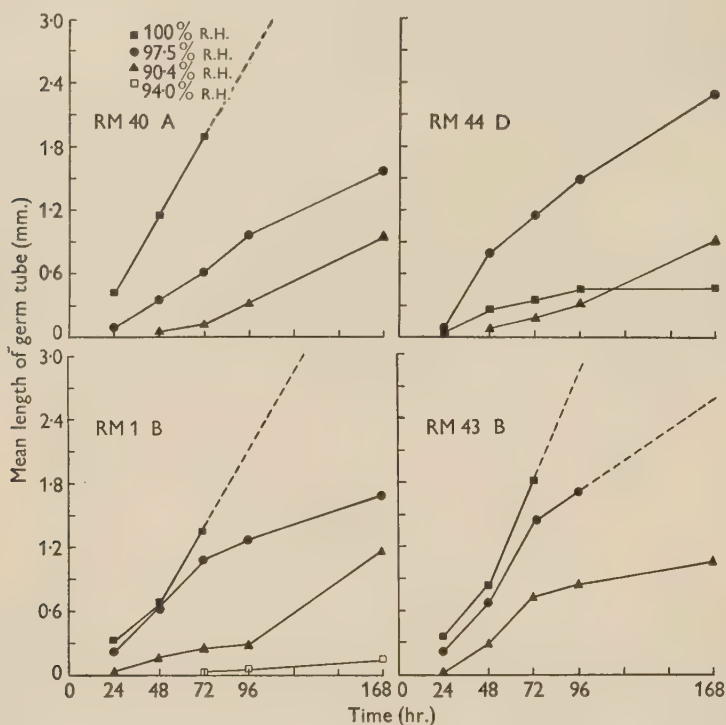
Field observations (Jones, 1953) suggested that strain B was limited biologically. Since it was known that the virulence of canker disease was affected by conditions of relative humidity in the micro-climate of a plantation, experiments were made to determine if the humidity of incubation had a differential effect on the mycelial growth and germination of conidia of the three strains.



Text-fig. 2. Effect of humidity on mycelial growth of strains of *M. unicornis* on P.D.A.

Mycelial growth. The three strains were grown on P.D.A. in the lids of glass jars. These lids had a ground glass fitting so that it was possible to expose the agar over mixtures of sulphuric acid to give atmospheres of varying relative humidity (Tomkins, 1932). After pouring the agar into the lids, 50 ml. of a specific acid mixture (Stevens, 1916) were added to each jar, the lids set in place and the jars then sealed and incubated for 7 days at 25° C. prior to inoculation. Text-fig. 2 shows the growth after 7 weeks. Growth of both A and B strains was limited in a saturated atmosphere, the optimal R.H. for A being 95 % and for B 90 %. With strain D there was no significant difference between the size of colonies at the four test humidities.

Germination of conidia. 1 mm. loopfuls of 2 % plain agar were dried on to cover-slips in a vacuum desiccator. Loopfuls of suspensions of conidia were dried on the agar spots and were exposed to atmospheres of different relative humidity controlled by sulphuric acid mixtures in van Tieghem cells. Percentage germination and extension of germ tubes were recorded at intervals after incubation at 20° C.



Text-fig. 3. Effect of humidity on germination of conidia of strains of *M. unicornis*.

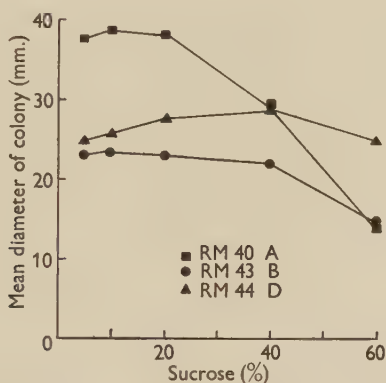
Text-fig. 3 shows the extension of germ tubes in collections of the three strains. With strains A and B growth was most rapid in a saturated atmosphere, although germination did not occur if there was heavy condensation and the conidia were submerged. Percentage germination normally rose to 100 % by 48 hr. Percentage germination and extension of germ tubes was less at 97.5 % R.H. for both strains and with strain A germination only started after 48 hr. Germination did not occur below 94.0 % R.H. with A but there was limited germination and germ tube extension with some collections of strain B (e.g. RM 1).

Strain D germinated best at 97.5 % R.H. and extension of germ tubes was limited in a saturated atmosphere. There was no germination below 94.0 % R.H.

Ascospores giving rise to strain A germinated exactly as strain A conidia.

Effect of the addition of sugar on growth on agar media

The addition of sucrose to agar media produces an effect on mycelial growth similar to that produced by lowering the humidity. Collections of the fungus were grown on P.D.A. plus 0.5 % yeast extract to which had been added 5, 10, 20, 40 and 50 % sucrose. The growth of colonies after 10 days at 20° C. is shown in Text-fig. 4 (means of five plates). The optimal level for the growth of A and B strains was between 10 and 20 % (differences between the levels were not significant). At 40 %, colonies were much smaller with strain A whilst with B there was only a slight decrease in size. Strain D grew best in 40 % sucrose.



Text-fig. 4. Effect of adding sucrose on mycelial growth of strains of *M. unicornis*.

Effect of the addition of sugar and salts on growth in liquid media

In amplification of the above experiments with agar media collections of the fungus have been grown in liquid media to which had been added sugar or salts.

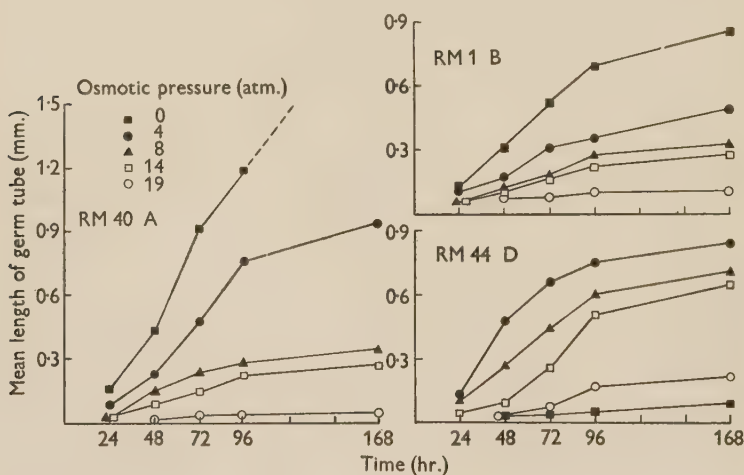
Media. A basal solution was prepared containing: K_2HPO_4 1.0 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., KCl 0.5 g., $FeSO_4 \cdot 7H_2O$ 0.01 g., urea 0.6 g., and yeast extract 0.6 g./l. of distilled water. In medium I, sucrose was added to the basal solution at five levels: 50, 100, 200, 300 and 400 g./l. In medium II, the urea of the basal solution was replaced by $NaNO_3$ 2.0 g., the yeast extract was increased to 5 g., and 30 g. sucrose per litre were added. Sodium and calcium chlorides in the proportion of 9 $NaCl$:1 $CaCl_2$ were added to this medium at five levels: 0.11M, 0.22M, 0.40M, 0.66M and 0.76M for each salt. The osmotic pressures of these solutions were taken or extrapolated from the data of Siminovitch & Briggs (1953).

The media were used in 100 ml. aliquots in 250 ml. Erlenmeyer flasks and incubation was at 20° C. without shaking. The inoculum consisted of five 2 mm. disks from the periphery of 10-day-old P.D.A. cultures for each flask of I and double this inoculum for each flask of II. Collections of the three strains were used and were harvested after 21 days for I and 16 days for II when the mycelia was washed carefully, dried, and weighed. The results are shown in Table 10. Strain A grew best in medium I at

5 % sucrose level and in medium II at the equivalent of a slightly higher osmotic pressure (7.3 atm.). The heavier growth in II was presumably attributable to higher yeast extract content. Strain B grew best in 20% sucrose and at approximately the same osmotic pressure (15.2 atm.) in II. As with A there was heavier growth in II. Strain D grew best in 10% sucrose in I but in 15.2 atm. in II. Compared with medium I, the dry weights of mycelium of D in medium II were less than those estimated from differences in the size of inoculum and period of incubation.

Table 10. *Effect of adding sucrose and salts on the growth of Monochaetia unicornis in liquid culture*

Osmotic pressure (atm.)	Sucrose (%)	I. Dry weight of mycelium (mg.) after 21 days at 20° C.			NaCl:CaCl ₂ 9:1 mixture	II. Dry weight of mycelium (mg.) after 16 days at 20° C.		
		RM 40A	RM 43B	RM 44D		RM 40A	RM 43B	RM 44D
3.5	5	61.6	119.2	242.1	0.11 M	143.7	180.4	130.9
7.3	10	56.2	120.2	330.1	0.22 M	162.1	182.4	139.0
15.2	20	48.0	145.5	215.9	0.40 M	152.7	230.6	149.6
23.7	30	37.4	90.8	216.2	0.60 M	143.0	201.9	137.5
32.5	40	24.5	81.3	201.0	0.76 M	123.4	176.7	99.2



Text-fig. 5. Effect of NaCl, CaCl₂, solutions of known osmotic pressure on germination of conidia of *M. unicornis*.

Germination of conidia. Mixed solutions of NaCl and CaCl₂ were used as the suspending media and 1 mm. loopfuls were placed in shallow depressions on microscope slides and sealed with cover-slips and petroleum jelly. The volume of liquid had to be small since submerged conidia will not germinate. For this reason counts and measurements were always made from conidia located at the edge of a droplet.

Results for the extension of germ tubes are shown in Text-fig. 5. Conidia of strains A and B grew best in water, the extension being comparable with that obtained over water in van Tieghem cells when the conidia had been dried directly on to the cover-slip without an agar surface. Germination

both strains was 100 % up to O.P. 8 atm. With strain A, germination reached 49 % at O.P. 14 atm. after 48 hr. and 32 % at O.P. 19 atm. after which there was no further germination. The same readings for strain B were 65 % and 15 %. Strain D germinated best at O.P. 4 atm. Percentage germination for the spore sample was only 44 % at this osmotic pressure being 13 % in water, 36 % at O.P. 8 atm., 21 % at O.P. 14 atm. and 10 % at O.P. 19 atm. There was no further growth at O.P. 19 atm.

With all strains the extension of germ tubes lessened with time and this was thought to be due to the partial submergence of the conidia and semi-aerobic conditions.

DISCUSSION

The experimental work has shown that there are good morphological distinctions in acervuli and conidia between the three strains A, B and D of *Monochaetia unicornis*. Collections of each strain show a range of variation, but in no instance does this mask strain differences. In the physiology of the three strains a distinction can be drawn between the 'parasitic' metabolism of collections of strain A which is associated with a virulent parasitism and the more 'saprophytic' metabolism of collections of strain B which is associated with mild parasitism. Strain D has proved to be even more 'saprophytic' and is unique in that it is known to be parasitic only on *Juniperus procera*.

Differences between the three strains have also been demonstrated in environmental conditions affecting mycelial growth, and germination of conidia. These differences may have some significance in the biology of their parasitism. Strains B and D are able to grow at lower humidities than strain A and they will tolerate higher sugar and salt concentrations. The fact that strain B was found only on the drier sites would suggest that it is only in these sites that it can compete with strain A. There are indications (Jones, 1953) that strain A is replacing strain B on the wetter sites. The typical habitat for *J. procera* also has a lower rainfall (25-35 in. annually) and the one collection of strain D was made in the rain shadow area of Mt. Kenya.

Wagener (1939) and Dimock (reported by Wagener, 1948) made a number of isolates from *Cupressus* species and *Sequoia sempervirens* of *Coryneum*, similar to *C. cardinale*, the causative agent of canker disease of cypresses in California. These isolates were found to be slower-growing on P.D.A., to have a lower optimal temperature for growth and to produce more conidia in culture than *C. cardinale*. They were weakly pathogenic or saprophytic and produced larger acervuli and slightly larger conidia than *C. cardinale*. With *Monochaetia unicornis*, many of these characters distinguish strain B from strain A. Dimock was able to show by monokaryotic cultures that variation was not the result of a heterokaryotic condition of the parent fungus, but no conclusion was reached as to whether these isolates comprised a single variable species or more than one species.

A considerable volume of work has been done on variation in pathogenic ascomycetes and the range of biotypes is often almost continuous in *Venturia inaequalis* (Keitt & Palmiter, 1938), in *V. pirina* (Stanton, 1953a, b)

and in *Diplocarpon rosae* (Frick, 1943). In some instances the genetic make-up of the isolates can be defined exactly, whilst in others there is strong evidence that the range is dependent on a heterokaryotic condition in the biotypes.

Loveless (1951) working with isolates of *Sclerotinia trifoliorum* derived from single ascospores and hyphal tips from sclerotia, found a reaction in most instances when two isolates were grown together on an agar plate which he described as a 'demarcation' line. He considered that these isolates comprised biotypes which had arisen by heterokaryosis. There was, however, a more pronounced reaction or 'incompatibility' line when an isolate of *S. trifoliorum* proper was grown together with an isolate of *S. trifoliorum* var. *fabae* and this phenomenon would appear similar to the antagonism between strains A and D, and B and D, of *Monochaetia unicornis*. The reaction between A and B strains appears similar to that of a 'demarcation' line. If it were possible to make detailed studies with the perfect stage of *M. unicornis* there would be more definite information on the status of the strains. As it is, A and B strains might be considered as biotypes of one species and strain D considered as a separate species or a variety (var. *juniperi*).

The present study has not elucidated the problem of the origin of the pathogen or the disease. Strain B has been found to sector freely in culture but the parent cultures and both A and D strains have remained extremely stable and virulent throughout 2-4 years in culture.

Part of the experimental work in this investigation was undertaken whilst holding a Post-doctorate Fellowship of the National Research Council of Canada, and I am most grateful to the Director and staff of the Prairie Regional Laboratory, Saskatoon, for their hospitality and assistance. I am indebted to Mr Peter Robinson for assistance with the statistical analyses and to Dr R. H. Haskins for reading and criticizing the manuscript.

REFERENCES

- CICCARONE, C. (1949). *Monochaetia unicornis* (C. et E.) Sacc., agente patogeno di un grave cancro dei Cipressi. *Ann. sper. agr.* **3**, 1-58.
- FRICK, LOUISE (1943). Untersuchungen über die Biologie und Pathogenität von *Diplocarpon rosae* (Lib.) Wolf. *Phytopath. Z.* **14**, 525-591.
- JONES, D. RUDD (1953). Studies on a canker disease of Cypresses in East Africa, caused by *Monochaetia unicornis* (Cooke & Ellis) Sacc. I. Observations on the pathology, spread and possible origins of the disease. *Ann. appl. Biol.* **40**, 323-343.
- JONES, D. RUDD (1954). Studies on a canker disease of Cypresses in East Africa, caused by *Monochaetia unicornis* (Cooke & Ellis) Sacc. III. Resistance and susceptibility of species of *Cupressus* and allied genera. *Ann. appl. Biol.* **41**, 325-335.
- KEITT, G. W. & PALMITER, D. H. (1938). Heterothallism and variability in *Venturia inaequalis*. *Amer. J. Bot.* **25**, 338-345.
- LOVELESS, A. R. (1951). The confirmation of the variety *fabae* Keay of *Sclerotinia sclerotiorum* Eriksson. *Ann. appl. Biol.* **38**, 252-275.
- RIDGWAY, R. (1912). *Color standards and color nomenclature*. Washington, D.C.
- SIMINOVITCH, D. & BRIGGS, D. R. (1953). Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. III. *Plant Physiol.* **28**, 15-34.
- STANTON, W. R. (1953a). Breeding pears for resistance to the pear scab fungus *Venturia pirina* Aderh. I. Variation in the pathogenicity of *Venturia pirina*. *Ann. appl. Biol.* **40**, 184-191.



1



4



2



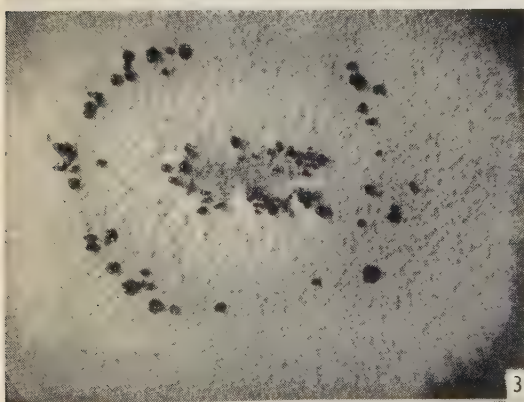
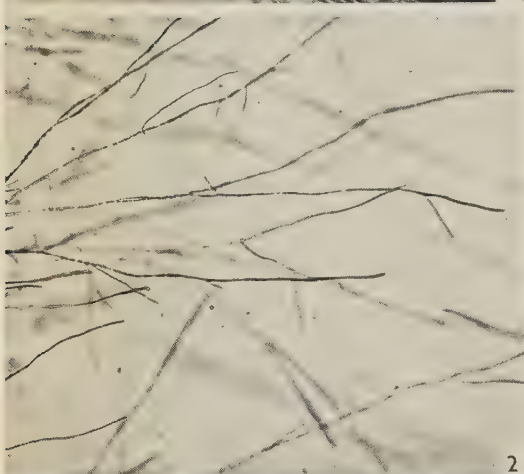
5



3



6



- ANTON, W. R. (1953*b*). Field variation in *Venturia pirina*. *Trans. Brit. mycol. Soc.* **36**, 90-103.
- EVENS, N. E. (1916). A method for studying humidity relations of fungi in culture. *Phytopathology*, **6**, 428-432.
- MCKINS, R. G. (1932). The action of certain volatile substances and gases on the growth of mould fungi. *Proc. Roy. Soc. B*, **111**, 210-226.
- AGENER, W. W. (1939). The canker of *Cupressus* induced by *Coryneum cardinale* n.sp. *J. agric. Res.* **58**, 1-46.
- AGENER, W. W. (1948). The New World Cypressess. Part II. Diseases of Cypressess. *El Aliso*, **1**, 255-321.

EXPLANATION OF PLATES 10 AND 11

PLATE 10

- gs. 1-3. Colonies of *Monochaetia unicornis* after 2 weeks at 20° C. on P.D.A. + 0.5 % yeast extract. Fig. 1. Strain A (RM 40). Fig. 2. Strain B (RM 1): blackening is due to masses of conidia. Fig. 3. Strain D (RM 44).
- gs. 4-6. Antagonism between strains of *M. unicornis* after 3 weeks at 20° C. on P.D.A. + 0.5 % yeast extract. Fig. 4. Two strain A collections (top RM 40; bottom RM 45). Fig. 5. Strain A and strain B (top RM 40; bottom RM 43). Fig. 6. Strain A and strain D (top RM 40; bottom RM 44).

PLATE 11

- gs. 1-3. Antagonism between strains of *M. unicornis* after 3 weeks at 20° C. on P.D.A. + 0.5 % yeast extract. (Continued.) Fig. 1. Opposing margins of strain A and strain D colonies. $\times 120$. Fig. 2. Strain A. Margin of colony without antagonism. $\times 120$. Fig. 3. *M. unicornis* (RM 35B) culture on P.D.A. after 4 weeks showing two rings of acervuli.
- gs. 4-6. Conidia of *M. unicornis* produced on P.D.A. + 0.5 % yeast extract. Fig. 4. Strain A (RM 40). Fig. 5. Strain B (RM 43). Fig. 6. Strain D (RM 44).

(Accepted for publication 11 November 1953)

MYXOMYCETES FROM JAMAICA*

BY CONST. J. ALEXOPOULOS AND E. S. BENEKE

*Department of Botany and Plant Pathology, Michigan State College,
East Lansing, Michigan, U.S.A.*

This paper lists thirty-eight species of Myxomycetes collected in Jamaica, B.W.I., from 29 June to 30 August 1952. Among those recorded is a species, *Comatricha martinii* Alexopoulos & Beneke, recently described as new by us from this collection, and a number of unusual or rarely collected species such as *Ceratiomyxa sphaerosperma*, *Physarum nicaraguense* and *Didymium intermedium*. Also included are *Licea biforis* and *Comatricha elegans*, which constitute new records from the American tropics, and a number of other species such as *Trichia affinis* and *T. favoginea* which are common in temperate regions, but which are said to be unusual in the tropics.

There are no publications which deal specifically with the Myxomycetes of Jamaica. The most extensive collections from the West Indies appear to have been made in Puerto Rico, Cuba, and the small islands of Antigua and Dominica, judging from published reports.

In recording the distribution of myxomycete species, Hagelstein (1944), Lister (1925), Martin (1949), and others, use general geographical designations such as 'throughout North America' or 'West Indies' without specifically mentioning Jamaica except in connexion with two or three species, and particularly *Alwisia bombarda* which has been found in the Western Hemisphere only on that island.

The Myxomycetes we are reporting below were collected in Jamaica, B.W.I., between 29 June and 30 August 1952. Most collections were made in the Blue Mountains and the Port Royal Mountains or their foothills in the parishes of St Andrew and Portland, the altitude of the specific localities varying from a few feet to 7400 ft. above sea-level.

Our headquarters were established at a pleasant old inn near Gordon Town, from which collecting trips were undertaken on foot or by car. The Institute of Jamaica at Kingston, and the Department of Botany, University College of the West Indies at Mona, offered facilities for tentative identification, for mounting specimens, and for moist chamber bark cultures.

For the first 10 days the weather in the south-eastern portion of the island was dry with little or no precipitation. After that frequent rains occurred, mostly in the afternoon. Such rains were often torrential, washing the slime mould fructifications off their substrata as they developed. Those that survived were attacked by moulds, insects and arachnids soon after they matured so that many of the collections consist of a very small number of fructifications, often no more than four or five, sometimes of but one.

An element of surprise and disappointment was introduced by the fact

* Contribution no. 53-14 from the Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

at very few plasmodia or fruiting bodies developed on bark from living trees, kept in moist chambers for 2-4 weeks. Of 221 pieces of bark from various species of trees, making up forty-four such cultures, only nineteen pieces in nine cultures gave any evidence of developing *Myxomycetes*, and only in seven of these cultures did mature fructifications develop. This is in marked contrast to our experience, and particularly to that of John E. Peterson (unpublished data), with cultures of bark from Michigan trees in which over 50 % of the bark specimens kept in moist chambers developed identifiable myxomycete fruiting bodies.

The list of species below includes a few specimens collected, during the period referred to above, by members of the staff of the Institute of Jamaica or the University College, which were added to our collection. The numbers recorded are those of our collection of *Myxomycetes* of Jamaica, except as otherwise noted. The names used are those accepted by Martin (1949) in his monograph.

We are grateful to Dr C. B. Lewis, director of the Institute of Jamaica, and to Prof. G. F. Asprey of the Department of Botany, University College of the West Indies, for laboratory facilities placed at our disposal; to Messrs G. R. Proctor, Botanist, and R. P. Bengry, Entomologist, both of the Institute, for their obliging co-operation throughout the progress of this work; and to Mr C. C. Gordon, of the Pathology Department, University College, for his valuable assistance in many ways. To our many Jamaican friends who made our brief visit to Jamaica so very enjoyable by their assistance, their hospitality, and their friendship, we express our deep gratitude.

Our sincere thanks go to Prof. G. W. Martin of the State University of Iowa, who identified a number of specimens and who confirmed some of our own identifications as credited, and to Dr D. P. Rogers of the New York Botanical Garden, who loaned us type and authentically determined specimens for comparison.

Our specimens have been added to the *Myxomycete* Collection of the Herbarium of Michigan State College. When material was available duplicate specimens were left at the Institute of Jamaica and at the Department of Botany, University College of the West Indies.

Family CERATIOMYXACEAE

Ceratiomyxa sphaerosperma Boedijn

No. 24; on dead herbaceous plant material (*Scybalium jamaicensis* ?); Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 11 July 1952.

This is a rather large collection of this interesting and unusual species. Martin (1949) states that the known distribution of this species is confined to the Panama Canal Zone, Costa Rica, and the East Indies.

Ceratiomyxa fruticulosa (Müll.) Macbr.

No. 4; on decaying wood; near Whitfield Hall, Blue Mts, St Thomas, elev. 4200 ft.; July 1952. No. 10; on old bark; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952. No. 20; on decaying wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 11 July 1952. No. 34; on

decaying wood; near Charlottenburg Estate, Port Royal Mts, St Andrew/Portland boundary, elev. 3500 ft.; 20 July 1952. No. 45; on decaying wood; Fern Gully, St Ann, elev. c. 750 ft.; 25 July 1952. Nos. 81, 83; on decaying wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952. No. 93; on decaying wood; by the public highway, below Newcastle, Port Royal Mts, St Andrew, elev. c. 2000 ft.; 16 August 1952. No. 98; on decaying wood; Dolphin Head, Hanover, elev. 1789 ft.; 20 August 1952.

This species occurs everywhere on the island. Most of the fruiting bodies collected were of the so-called *flexuosa* type which appears to be particularly prominent in tropical regions.

Family LICEACEAE

Licea biformis Morgan

No. 30; on the outer surface of bark; near Bath, St Thomas, on the road between Bath and Bath Fountain, elev. 100 ft.; 15 July 1952.

This collection consists of only two sporangia. They occur together with two sporangia of the yellow form of *Arcyria cinerea* (No. 30a). In the western hemisphere this minute species has been found in Ontario, in some of the northern parts of the United States and in Kansas. This is, therefore, the first record of this species from the American tropics. As Hagelstein (1944) points out, the species is probably universally distributed, but has been overlooked because of its minute size.

Family CRIBRARIACEAE

Cribraria violacea Rex

No. 57; developed in moist chamber culture on bark collected on 2 July 1952 above Gordon Town, St Andrew, on road to Newcastle, elev. c. 2000 ft. Nos. 60, 67a; on decaying wood; near Four Foot, Portland, on path from Milbank, elev. c. 750 ft.; 11 August 1952. No. 111; developed in moist chamber culture on bark collected on 2 August 1952 near Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.

The last development (no. 111) consists of but a single sporangium.

Cribraria tenella Schrad.

No. 26; on decaying wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 11 July 1952. No. 82; on decaying wood, Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952.

No. 26 is a small collection of but a few sporangia, a number of which are mouldy. The sporangia measure 0.3 mm. or less in diameter, the cup is small and rudimentary, the dark granules of the net are oval or elongated. A comparison with authentically determined material (New York Bot. Gard. No. 2139) shows our sporangia to be much smaller and the spores to be distinctly warted or spinulose rather than indistinctly so to almost smooth as in the authentic material. These characters suggest a relationship to *C. microcarpa*. In their discussion of *C. microcarpa*, Macbride & Martin (1934) state that the entire sporangium of this species assumes a violet colour when mounted in KOH. Since no such colour change could be detected in our material, it seems preferable to consider this collection as representing a variation of *C. tenella*. No. 82 is a more typical example of *C. tenella* than the preceding.

Cribraria microcarpa (Schrad.) Pers.

No. 38; on decaying wood; near Charlottenburg Estate, Port Royal Mts, St Andrew, elev. 3500 ft.; 20 July 1952.

This collection consists of a few rather typical sporangia of this species. The fructifications assume a purple colour when immersed in 10 % KOH.

Cribraria splendens (Schrad.) Pers.

Nos. 9, 11; on decaying wood; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952.

Dictydium cancellatum (Batsch) Macbr.

On decaying wood; west slope of Blue Mt, St Andrew, elev. 6000–7000 ft.; collected by G. R. Proctor as no. 7061; 22 July 1952.

Family TRICHIACEAE

Perichaena depressa Libert.

No. 51; on decaying wood; near Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 31 July 1952.

Perichaena chrysosperma (Currey) Lister

No. 72; on old bark; near Four Foot, Portland, on the path from Milbank, elev. c. 750 ft.; 11 August 1952.

A very small collection.

Arcyria cinerea (Bull.) Pers.

No. 19; on decaying wood, Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952. Nos. 30a, 32; on old bark; near Bath, St Thomas, on the road to Bath Fountain, elev. 100 ft.; 15 July 1952. No. 35, on old bark, no. 37, on decaying wood; near Charlottenburg Estate, Port Royal Mts, St Andrew, elev. 3500 ft.; 20 July 1952. G. R. Proctor no. 7066; on decaying wood; summit area, Blue Mt, elev. 7000–7400 ft.; 22 July 1952. Nos. 66, 67, 69, 74; on old bark; near Four Foot, Portland, on the path from Milbank, elev. c. 750 ft.; 11 August 1952. No. 87; on old bark; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952. Nos. 95, 97a; on decaying bark; by public road, below Newcastle, Port Royal Mts, St Andrew, elev. c. 2000 ft.; 16 August 1952. No. 104; on decaying wood; near Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. c. 4000 ft.; 24 August 1952. No. 108; on decaying wood; Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. 4800 ft.; 24 August 1952.

This is probably the most widely occurring of the Myxomycetes in Jamaica. Both the typically grey form (nos. 19, 37, 42, 66, 67, 87, 95, 104, 108) and the light yellow form (nos. 30a, 32, 35, 67, 69, 97a) are abundant. No. 32 was identified by Dr G. W. Martin.

Arcyria denudata (L.) Wettst.

Nos. 27, 28; on decaying wood; near Bath, St Thomas, on road to Bath Fountain, elev. 100 ft.; 15 July 1952. No. 44; on old bark; at the 7-mile post between Barrett's Gap and Corn Puss Gap, St Thomas, elev. c. 1500 ft.; collected by R. P. Bengry; 25 July 1952. No. 46; on dead leaf; west slope of John Crow Mts, elev. 2500 ft.; collected by G. F. Asprey; 26 July 1952. No. 47, on old coconut husk, no. 49, on old log;

both near Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 31 July 1952. Nos. 65, 68; on decaying wood; near Four Foot, Portland, on path from Milbank, elev. c. 750 ft.; 11 August 1952. No. 94; on decaying wood; by the public road, below Newcastle, Port Royal Mts, St Andrew, elev. c. 2000 ft.; 16 August 1952.

This species is almost as common as *A. cinerea*, and is as widely distributed over the island.

***Arcyria insignis* Kalchbr.**

No. 22; on decaying wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 11 July 1952.

A small collection of but a few sporangia.

***Trichia affinis* de Bary**

No. 5; on old bark; near Whitfield Hall, Blue Mts, St Thomas, elev. 4200 ft.; 5 July 1952.

The spores measure 10–11.3 μ and are, therefore, of somewhat smaller dimensions than given in the descriptions of this species. There are a few substipitate sporangia among the sessile ones. This species is found typically in temperate regions and is 'less common in the tropics' according to Martin (1949). Specimen identified by Dr G. W. Martin.

***Trichia favoginea* (Batsch) Pers.**

Nos. 8, 13, on old wood, no. 17 on old bark; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952; identified by Dr G. W. Martin.

This species, like the previous one, is very common in the temperate regions, but is said not to be common in the tropics.

***Trichia verrucosa* Berk.**

No. 12; on decaying wood; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952.

A small collection of a few imperfect, but definitely identifiable, sporangia.

***Hemitrichia serpulula* (Scop.) Rost.**

No. 7; on decaying wood; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 7 July 1952. No. 103; on decaying wood; near Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. c. 4000 ft.; 24 August 1952. No. 109; on old bark; Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. 4800 ft.; 24 August 1952. No. 110; on old bark; near Cinchona, Blue Mts, St Andrew, elev. 4800 ft.; 24 August 1952.

***Hemitrichia stipitata* (Masse) Macbr.**

No. 6; on old bark; near Whitfield Hall, Blue Mts, St Thomas, elev. 4200 ft.; 5 July 1952. Nos. 16, 18; on decaying wood; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952. G. R. Proctor no. 7067; on decaying wood; summit area of Blue Mt, elev. 7000–7400 ft.; 22 July 1952. No. 56; on decaying wood; Fern Gully, St Ann, elev. c. 750 ft.; 25 July 1952. No. 63; on decaying wood; near Four Foot, Portland, on path from Milbank, elev. 750 ft.; 11 August 1952. No. 92; on decaying bark; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952. No. 101; on decaying wood; Hardwar Gap, Port Royal Mts, St

Andrew/Portland boundary, elev. 3900 ft.; collected by R. P. Bengry, 24 August 1952. No. 105; on old bark; near Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. c. 4000 ft.; 24 August 1952.

A very common species occurring all over the island.

Hemitrichia vesparium (Batsch) Macbr.

No. 39; on old bark; near Charlottenburg Estate, Port Royal Mts, St Andrew, elev. 3500 ft.; 20 July 1952.

Family ECHINOSTELIACEAE

Echinostelium minutum de Bary

No. 59; developed in moist chamber culture on bark of *Pinus massoniana*, collected on 7 July 1952 on the grounds of Whitfield Hall, Blue Mts, St Thomas, elev. 4200 ft.

Numerous sporangia of the pink form developed in this culture, in sharp contrast to the pure white and somewhat larger form which, in the senior author's experience, usually develops on pine bark from Florida, U.S.A., but in common with developments usually obtained on bark of various Michigan trees.

Family STEMONITACEAE

Diachea leucopodia (Bull.) Rost.

No. 80; on living herbaceous plant stems; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952.

Stemonitis nigrescens Rex

No. 52; on decaying wood; on road from Bath, St Thomas, to Corn Puss Gap, between the 5- and 6-mile posts, elev. c. 500 ft.; 1 August 1952. No. 90; on old wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 16 August 1952.

According to Hagelstein (1944) *S. nigrescens* is only a variety of *S. fusca*, differing from the latter by its small developments, its small sporangia, and its larger spores. Martin (1949) recognizes two distinct species, however, and separates them chiefly on size of cluster and size of sporangia. He gives practically the same spore measurements for both species. In our specimens, the reticulate pattern of the spines on the spores is not always clear, and the spores measure $9.7-11.3 \mu$ as contrasted to a maximum of 9μ given by Martin for this species.

Comatricha elegans (Racib.) Lister

No. 3; on burned wood; near Whitfield Hall, Blue Mts, St Thomas, elev. 4200 ft.; 5 July 1952. No. 48; on old coconut husk; near Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 31 July 1952. Nos. 61, 73, 77; on old wood; near Four Foot, Portland, on path from Milbank, elev. c. 750 ft.; 11 August 1952.

This species does not appear to have been reported previously from the American tropics. Collection no. 48 is a rather large development for this species, consisting of a few hundred sporangia, whereas the other four collections consist of two or three sporangia each. The spores of no. 73 measure $6.5-10 \mu$, representing a wider range than given in the descriptions.

Comatricha martinii Alexopoulos & Beneke

No. 78; developed in moist chamber culture on bark of unidentified tree, collected on 26 July 1952 on the property of Eaton Hall Hotel, Runaway Bay, St Ann, elev. 15 ft.

This is the type collection of this species, recently described by us (Alexopoulos & Beneke, 1954).

Comatricha typhoides (Bull.) Rost.

No. 36; on decaying wood; near Charlottenburg Estate, Port Royal Mts, St Andrew, elev. 3500 ft.; 20 July 1952. No. 53; on decaying wood; on road from Bath, St Thomas, to Corn Puss Gap, between the 5- and 6-mile posts, elev. c. 1250 ft.; 1 August 1952. No. 100; on decaying wood; Hardwar Gap, Port Royal Mts, St Andrew/Portland boundary, elev. 3900 ft.; 23 August 1952.

Clastoderma debaryanum A. Blytt

G. R. Proctor, no. 7061a; on decaying wood; summit area of Blue Mts, elev. 7000-7400 ft.; 22 July 1952. Nos. 106, 107; on decaying wood; near Morse's Gap, Blue Mts, St Andrew/Portland boundary, elev. c. 4000 ft.; 24 August 1952. The Proctor collection consists of but a single sporangium.

Lamproderma arcyriionema Rost.

Nos. 29, 32; on bark of decaying log; near Bath, St Thomas, on the road to Bath Fountain, elev. 100 ft.; 15 July 1952. No. 96; on bark of decaying log; Hardwar Gap, Port Royal Mts, St Thomas/Portland boundary, elev. 3900 ft.; 16 August 1952. All identified by Dr G. W. Martin.

Family **PHYSARACEAE****Physarum nicaraguense** Macbr.

Nos. 1, 2; on bark of dead tree; Mamee River bed, back of Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 29 June 1952. Identification confirmed by Dr G. W. Martin.

Physarum bogoriense Racib.

No. 15; on decaying wood; Portland Gap, Blue Mts, St Thomas/Portland boundary, elev. 5500 ft.; 6 July 1952.

Physarum leucophaeum Fries

No. 50; on bark of decaying log; near Mr Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 31 July 1952.

Physarum stellatum (Masse) G. W. Martin

No. 58; developed in moist chamber culture on bark from living tree, collected above Gordon Town, St Andrew, on the road to Newcastle; 2 July 1952; identification confirmed by Dr G. W. Martin. No. 71; on dead twig; near Four Foot, Portland, on path from Milbank, elev. c. 750 ft.; 11 August 1952. No. 86; on old bark; Hardwar Gap, Port Royal Mts, St Thomas/Portland boundary, elev. 3900 ft.; 16 August 1952. Both identified by Dr G. W. Martin.

Physarum viride (Bull.) Pers.

No. 62; on old wood; near Four Foot, Portland, on path from Milbank, elev. c. 750 ft.; 11 August 1952.

Physarum nucleatum Rex

No. 97; on decaying wood; by the public road, below Newcastle, Port Royal Mts, St Andrew, elev. c. 2000 ft.; 16 August 1952.

The spores in this collection measure 8–10 μ , mostly 8.5 μ . This is somewhat larger than the size reported in the literature (7–8 μ), but other characteristics are typical of the species.

Physarum (?) pusillum (Berk. & Curtis) G. Lister

No. 23; on old wood; Hardwar Gap, Port Royal Mts, St Thomas/Portland boundary, elev. 3900 ft.; 11 July 1952; identified by Dr G. W. Martin.

Although not typical of *P. pusillum*, this collection resembles this species more than any other. It differs from typical *P. pusillum* in that: the peridium is iridescent brown-bronze under the lime; there is little evidence of a cup-like base in the sporangium; and the spores are much smaller, measuring 7.6–8.1 μ , as contrasted to 10–12 μ for the typical form. We are following Dr Martin's suggestion to place it in this species tentatively.

Physarella oblonga (Berk. & Curt.) Morgan

No. 55; on wood of dead tree; near Mt Mansfield Inn, Gordon Town, St Andrew, elev. 1000 ft.; 2 August 1952.

Family DIDYMIACEAE

Didymium clavus (Alb. & Schw.) Rab.

No. 25; on bark of decaying log; Hardwar Gap, Blue Mts, St Thomas/Portland boundary, elev. 3900 ft.; 11 July 1952.

Didymium nigripes (Link) Fries

Nos. 84, 85, 91, on living leaf and stems of herbaceous plants; nos. 88, 89, on plant debris; Hardwar Gap, Blue Mts, St Thomas/Portland boundary, elev. 3900 ft.; 16 August 1952.

Didymium intermedium Schroet.

No. 99; on bark of decaying log; Hardwar Gap, Blue Mts, St Thomas/Portland boundary, elev. 3900 ft.; 23 August 1952; identified by Dr G. W. Martin.

REFERENCES

- ALEXOPOULOS, CONST. J. & BENEKE, E. S. (1954). A new species of *Comatricha* from Jamaica. *Mycologia*, **46**, 245–247.
HAGELSTEIN, R. (1944). *The Mycetozoa of North America*, 306 pp., 16 pls. Mineola, N.Y.: published by the author.
LISTER, A. (1925). *A Monograph of the Mycetozoa*, ed. 3 (revised by Gulielma Lister), xxxii + 296 pp., 222 pls. British Museum (Nat. Hist.). Oxford University Press.
MACBRIDE, THOMAS H. & MARTIN, G. W. (1934). *The Myxomycetes*. ix + 339 pp., 21 pls. New York: The Macmillan Company.
MARTIN, G. W. (1949). *Myxomycetes. North American Flora*, vol. 1, pt. 1, 190 pp. New York: New York Bot. Gard.

(Accepted for publication 23 November 1953)

DEME STRUCTURE IN HIGHER FUNGI: *MYCENA GALOPUS*

By A. F. PARKER-RHODES

University of Cambridge

Collections of *Mycena galopus* (Pers. ex. Fr.) Kummer have been made in several woods and plantations in south-east Suffolk and one locality in Essex; spore samples have been taken from these, measured and analysed statistically.

The results are tabulated, so as to show for each mycelium sampled the mean length and mean shape of its spores, and the third relative cumulant of the distribution of length, measured by a specified function of the original data. In the three cases where more than one basidiocarp was taken from one mycelium there was no evidence for polydiplontism; this was as expected in view of the quisquicolous habit of the species.

Despite many significant differences between mycelia, there were none that could be attributed to some being heterozygous relatively to others. In so far as anything can be inferred from this, it favours the view that the species is at least facultatively homothallic.

The number of combinations of values of the three factors tabulated was significantly less than would be expected, if there were no genetically determined phenodemes distinguishable by particular combinations. It was concluded that the species is in fact polymorphic in this respect. No correlation of these phenodemes with locality, or with the named colour variants of the species, was found (though for the latter the data are too few to be indicative). This suggests an absence of selective advantage for any phenodeme in any of the localities.

It was concluded that the higher fungi may show a similar deme structure to that known in animals and plants generally. The possible practical implications of this are mentioned.

INTRODUCTION

Much information has been collected by many workers on the fine structure of those elusive entities called species in animals and in plants. But up to now little has been published on population structure in the higher fungi. For a proper understanding of the ecology of the fungi, and in particular to assess the role of a given binome in the plant associations where it occurs, it is necessary to know something of the biological status of the group of individuals bearing the name, of what sort of units it is built up, and of the relations between these units. Anything approaching a complete answer to these questions would require the application of a wide variety of techniques; but just as much has in the past been learned about the structure of species of flowering plants by statistical measurements on collections of specimens, so we may expect to be able to make some progress by the same methods in the higher fungi.

A species (by which I mean a binome, that is a group of individuals to which is assigned a given binomial) may be regarded as made up of a number of demes, in the sense of Gilmour and Heslop-Harrison (1954), of various kinds and categories; I shall assume that the reader is familiar with this deme terminology. The problem in general is to map, in as much detail

is required, all the demes of the species studied of each kind which can be discovered in a given area. The smaller the area, the more detailed the analysis can be. For purposes of ultimate practical application, the most important thing arising out of such study will be a knowledge of the nature of the ecological and geographical barriers within the species. Attention must be directed more upon the boundaries between them than on the demes themselves.

MATERIAL

The present study concerns a single species, *Mycena galopus* (Pers. ex. Fr.) Kummer, in a small area in south-east Suffolk and one locality in Essex. The species was chosen mainly on account of its abundance in the area, and for being a species known to exhibit considerable variability in its ordinary taxonomic characters. The localities in which it was collected were a series of woods and plantations conveniently accessible from the Flatford Mill field centre of the Council for the Promotion of Field Studies.

M. galopus is a quisquicolous woodland species, which shows little preference for any particular kind of forest association in this part of Britain; it occurs with apparently equal frequency in coniferous and deciduous woods, and is also found, though less commonly, in fields and hedgerows. With the exception of the estuaries which intersect the region, and which are nowhere more than a mile in width, there is no part of the area which can be regarded as uninhabitable for *M. galopus*, though the population density must be much higher in the small and widely scattered woods than in the intervening country. It was therefore uncertain whether there would be any appreciable genetic isolation of one wood from another, though theoretical considerations suggested that there would not be. In addition to the typical form, three named varieties were also collected in small numbers. These are: var. *alba*, in which the pileus is almost wholly white instead of the typical pattern with dark greyish centre and pale striate margin; var. *nigra*, uniformly fuscous grey; and var. *olivacea*, in which the dark grey is replaced by olive green. The taxonomic value of these names is very dubious, and the present investigation has done nothing to enhance it.

The localities where the collections were made were as follows:

Fishpond Wood, near Flatford: a small copse mainly of *Fraxinus excelsior*, with *Sambucus nigra* and other shrubs. Specimen No. Mgp. 52 FF. 1.
Dodnash Wood: a plantation containing *Quercus robur*, *Castanus sativa*, and other trees, but much neglected and in parts much overgrown with shrubs (*Corylus*, *Prunus*, etc.) and bracken (*Pteridium aquilinum*), and in swampy places with *Alnus glutinosa*. Mgp. 52 FF. 2, 5, 6, 7, 8, 9.
Stour Wood, near Manningtree, Essex: a large plantation almost wholly of *Castanus sativa* in coppice. Mgp. 52 FF. 3, 4.
Assington Thicks: a felled plantation spontaneously recolonized by *Betula alba*, *Fraxinus excelsior* and *Quercus robur*, but still actively developing and containing open spaces with *Ulex europaeus*, *Calluna vulgaris*, and *Pteridium aquilinum*. Mgp. 52 FF. 10, 12, 13, 14.

East Bergholt Plantations: containing a wide variety of angiospermous and coniferous trees. Mgp.52 FF.15 (var. *alba*), 16 and 17 (var. *olivacea*).

Staverton Thicks: a portion of ancient forest containing little else than *Quercus robur*, *Ilex aquifolium*, and *Pteridium aquilinum*. Mgp.52 FF.18-22 (type) and 23 (var. *nigra*).

Hollesley Heath: an area of heathland due to Iron-age deforestation, with modern plantations of *Pinus sylvestris*. Mgp.52 FF.24 collected near to one of these.

METHODS

Quantitative observations were made only on the spores; only the spores can be obtained in sufficient numbers for adequate statistical analysis, and there are few macroscopic characters of the higher fungi which are under sufficiently autonomous morphogenetic control to yield results from which one can infer anything of the genotype. The specimens for study were collected in the field, and spore prints were taken from them during the following night on glass slides. In most cases only one basidiocarp was taken from any one fructifying mycelium, but in a few two or more were taken, in order to ascertain whether this species might exhibit polydiplontism, as recorded by the author for *Hygrocybe turunda* (Parker-Rhodes, 1954) and probably of common occurrence in humicolous Basidiomycetes.

For subsequent measurement, a sample of spores was taken from the spore print with a flattened needle, transferred to a clean slide, and immersed in very dilute aqueous safranin, the excess being drained off after placing the cover-glass. After allowing 2 min. for staining, the spores were measured under an oil-immersion lens giving a magnification of about 1300 diameters; in each sample 100 spores were measured, the usual precautions being taken to avoid duplication and bias. The measurements made were of the greatest length and greatest breadth of each spore, each being recorded to the nearest odd quarter of a micrometer division, the actual grouping interval being approximately 0.5μ . From four specimens, nos. 1, 2, 10 and 24, insufficient spores were obtained to yield a measurable sample.

The spores are elliptical, thin-walled, and hyaline, with a refractive index not greatly different from that of water so that they are not easy to see without staining. There is a small apiculus, but no discernible germ-pore. The average measurements over the whole series of observations were $9.54 \times 5.17 \mu$.

In the statistical analysis, the two measurements made on each spore were represented by the length and the shape-ratio, that is, ratio of length to breadth. For each of these, the procedure was as follows. First, the mean $\bar{x}$ and standard deviation $\bar{s}$ were calculated for each specimen (after it had been found that there were no significant differences between different basidiocarps from a single mycelium, figures from these were pooled without further adjustment). Using these estimates, the raw variates x were replaced by the standardized variates $(x - \bar{x})/\bar{s} = x'$. The whole of the data, transferred to the standardized variates, was then

pooled by adding together the numbers observed in all the samples within each of a series of ranges of the standardized variate centred on integral and half-integral values. This change of grouping introduces a small additional random error which has been ignored. An empirical normalizing function was then found, by graphical methods applied to these pooled figures, which, operating on the standardized variate, would transform it into a normal variate. The third, fourth and fifth relative cumulants were then calculated for each of the original samples, using the normalized variates. No regrouping was made at this stage, so that the final group widths were unequal.

Finally, a table was prepared showing all the relevant statistics for both measurements on all the samples, from which it could be ascertained whether any significant classification of the specimens could be made on this basis.

RESULTS

The normalizing functions selected were for both measures (length and shape ratio) of the form

$$y = \sqrt{(a + bx')} - c$$

(where $x' = (x - \bar{x})/\bar{x}$). It was somewhat surprising that the same form was applicable to both length and shape; neither of the functions used in the application of the same technique of *Hygrocybe turunda* (Parker-Rhodes, 1954) could be fitted to these data. The values of the parameters which gave the best fit, as found graphically, were:

	<i>a</i>	<i>b</i>	
For lengths	29	10	0
For shape ratios	53	14	1.96

In the table of results (Table 1), a distribution is said to be 'normal' when it is of the form defined by this function, and deviations from normality are to be interpreted accordingly. It was found that there were no significant departures from normality in this sense in regard to shape ratio, so that only the mean shape ratios are recorded in the table. Two specimens, Mgp. 52 FF. 17 and 23, yielded spores of so anomalous a shape that they were disregarded as having probably suffered maltreatment.

INTERPRETATION

It has already been mentioned that no significant differences were found in any of the parameters of distribution between the spore samples from different basidiocarps from one mycelium. The samples nos. 4-6 in the above table accordingly contain 200, 400 and 200 spores each, instead of 100 as do all the others, since the collections included 2, 4 and 2 basidiocarps from these mycelia. This evidence, so far as it goes, does not suggest the occurrence of caryallaxis (secondary interchange of nuclei between dicaryons of already binucleate mycelia) in *Mycena galopus*. This is indeed to be expected, in view of the necessarily restricted spread of a given binucleate mycelium in a quisquicolous species, inhabiting twigs and small fragments of dead wood rather than large logs or the indefinitely extended leaf mould of the forest floor.

Table 1. *Statistics of spore measurements in Mycena galopus*

Group	No	Lengths			Shapes		Locality
		Mean	S.D. of mean	Third relative cumulant	Mean	S.D. of mean	
A	3	40.2	0.335	+0.63	1.791	0.0116	Stour Wood
B	5	35.7	0.225	+0.31	1.983	0.0116	Dodnash
	6	37.0	0.336	+0.35	1.842	0.0167	Dodnash
	9	36.0	0.347	+0.28	1.970	0.0157	Dodnash
	14	35.7	0.417	-0.18	1.805	0.0174	Assington
	18	35.7	0.421	+0.09	1.938	0.0354	Staverton
Co	4	33.9	0.139	-0.20*	1.664	0.0070	Stour Wood
C1	7	34.0	0.283	+0.27**	1.881	0.0129	Dodnash
	12	33.1	0.297	-1.67	1.812	0.0158	Assington
	20	34.5	0.348	-0.54	1.905	0.0165	Staverton
C2	15	34.4	0.338	-0.50	2.034	0.0223	E. Bergholt
	16	34.3	0.356	-0.54*	2.085	0.0261	E. Bergholt
D	8	33.4	0.314	+0.29	1.851	0.0161	Dodnash
	13	33.6	0.304	-0.14	1.714	0.0144	Assington
	19	32.1	0.254	-0.04	1.722	0.0138	Staverton
	21	33.9	0.367	+0.33	1.722	0.0178	Staverton
	22	32.5	0.300	-0.09	1.741	0.0172	Staverton
	17	30.4	0.285	+0.04	1.482	—	E. Bergholt
	23	35.5	0.284	-0.09	1.286	—	Staverton

Mean lengths are given in units of 0.275μ . Figures given in *italic* are significant at 5%.

* Fourth cumulant significantly positive. ** Fifth cumulant significantly positive.

There is no evidence from the higher cumulants that any of the distributions can be interpreted as resultants by crossing of two or more of the other distributions; if there are such cases, they do not show up on an analysis of the spore prints, though they might have done so had the spores been measured in their tetrads on the gill face, a thing difficult to do with hyaline spores such as these. It would indeed be surprising if there were really no heterozygosity in factors affecting the size and shape of the spores; the result could be accounted for either by the relatively large numbers of genetic factors involved, or, which is perhaps more in accordance with the observations, by the basidiocarps being mainly or entirely homothallic in origin.

In Table 1 the seventeen classified samples have been divided into six groups. This grouping is made as follows:

Group	Length (in units of 0.275μ)	Distribution of length	Shape
A	Over 39	Positively skew	Intermediate
B	38-35	Normal	Narrow
Co	35-33	Non-normal, mainly negatively skew	Very broad
C1			Intermediate
C2			Very narrow
D	Under 34	Normal	Broad

If exception is taken to the relatively narrow spores of Mgp. 52FF.8, which I have placed in group D, these six groups could be increased to seven. They are based on a fourfold division of length and shape, and

a threefold division of length distribution. Length and distribution of length alone yielded twelve classes of which four are realized; if the grouping were due entirely to chance, the observed distribution of class size would be as unequal as this in less than 5% in replicates, and may therefore be regarded as significant. Length and shape alone yield sixteen classes, of which six are not empty; this is only significant to 7%. All three criteria together yield thirty-six classes, and the grouping is now very highly significant.

This shows that there is a real polymorphism in *M. galopus* in this area, which is revealed by considering the length and shape of spores and their distribution functions as taxonomic characters. It seems reasonable to imagine that a minuter analysis, employing for example much larger spore samples, could have revealed yet nicer distinctions. On the other hand, the five localities represented, with the six groups already delimited, define thirty classes of which twelve are represented among only seventeen individuals, which suggests a random assortment of the morphological groups among the localities. That, despite this geographical arrangement, the groups are distinguishable, suggests that they have a genetic basis and are not mere phenotypes reflecting some environmental condition. Indeed it seems to be a general rule, that statistical spore characters such as these are usually dependent more on genetical than on environmental factors; which rule, if it could be more firmly established, would be a very useful one in micro-taxonomy.

DISCUSSION

These observations seem to show that there exists in *M. galopus*, at least in the small district of Britain studied but presumably elsewhere also, a system of genetically determined phenodemes which may recur, so far as can be yet seen, in any locality where the fungus grows. Presumably they are determined by the recombination of a relatively small number of genes. The entities concerned are based morphologically on distinctions which are purely statistical; that is to say, although the differences may be 'significant' in the technical sense, it is impossible to assign a given specimen to one or another of the phenodemes merely by measuring a few spores under the microscope, as one does in routine identifications. On this account, it is inadmissible to regard them as nameable varieties; varieties have been named in this species, mainly on colour differences, and these varieties have been found during this study; but the phenodemes cut across them. Perhaps indeed these phenodemes are of more taxonomic significance than the varieties based on colour; but that is rather an argument for ignoring the latter than for naming the former.

The failure of the phenodemes to show any significant segregation between different topodemes is in accordance with expectation, if the differences concerned have no selective advantage one over another. For the woods and plantations which form the ecological background of the topographical pattern of the neighbourhood are (with one exception) of quite recent origin, so that there can hardly have been time, even if the degree of isolation between one locality and another is much greater than appears likely, for random mutations to accumulate to the point of pro-

ducing observable non-adaptive differences. There is reason to expect that in small isolated habitats there will be some selective advantage in large spores, as being less readily dispersed to great distances and so lost to the habitat; but calculation shows that even if all the numerical factors involved have their optimum values such selection should produce a detectable effect only after periods of the order of 1000 years, and since gross changes of climate have occurred since less than 10,000 years ago it is scarcely possible that the effects of such selection will be detectable in any part of the British Isles to-day.

However, it is still probable that if sufficiently extensive data were available, a difference in the *proportion* of the different phenodemes could be detected as between one topodeme and another. Differences of this kind would require collections of some hundreds of specimens to be demonstrated statistically; but they would presumably be magnified by distance, so that over a wide area, say the whole of Britain, different phenodemes could be shown to have perceptibly different distributions, as is the case with many polymorphic animals and plants. Such an investigation, applied to species of more economic importance than *M. galopus*, might well give valuable information on the deme structure and sub-specific organization of the higher fungi.

The results here reported show that, despite differences in their mode of reproduction and gene exchange, the fungi may have a similar species structure to that usual in animals and higher plants. If this is so, a wide field for future research exists in this line, and many questions of great practical interest are raised. For example, how much opportunity exists for gene-exchange between individuals of a mycorrhizal species attached to different species of tree, or different individual trees? There may be no barriers, or on the other hand there may be distinct physiologic races, and on the answer depend many questions regarding the introduction of mycorrhizasts into new plantations. Again, we shall need to investigate the precise relationship between the parasitic and saprophytic phases of fungi such as *Armillaria mellea* which can attack living trees as well as dead wood. It is one thing to discover what is *possible*, by laboratory experiments, but quite another to work out how these possibilities actually work out in nature, which is usually what most closely concerns us.

I wish to thank the Warden and staff of the Flatford Mill Field Centre for the use of their laboratory facilities, and Captain Wake-Walker for permission to collect specimens from his estate.

REFERENCES

- GILMOUR, J. S. L. & HESLOP-HARRISON, J. L. (1954). The deme terminology and the units of micro-evolutionary change. *Genetica* (in the Press).
PARKER-RHODES, A. F. (1954). The Basidiomycetes of Skokholm Island. XI. Intramycelial variation in *Hygocybe turunda* var. *lepida*. *New Phytol.* **53**, 145.

(Accepted for publication 24 November 1953)

THE BRITISH MYCOLOGICAL SOCIETY TRANSACTIONS

PART

Vol.	1	2	3	4	5
1	m	m	6s.	m	m
2	[		68s.		]
3	[		80s.		]
4	m	m	—	—	—
5	m	m	m	—	—
6	m	m	(20s.)	)	—
7	(	40s.		)	—
8	(	40s.		)	—
9	(	20s.	)	m	—
10	(	40s.		)	—
11	[	40s.		]	—
12	(10s.)	m	m	(10s.)	—

PART

Vol.	1	2	3	4
13	m	m	m	m
14	[	40s.		]
15	m	m	m	m
16	m	[	30s.	]
17	m	m	[10s.]	m
18	m	[	30s.	]
19	m	(	30s.	)
20-29	(	40s.		)
30	(	Jubilee Vol.	40s.	)
31	[	25s.	]	m
32	[	25s.	]	m
33-36	(	50s.		)

m = out of print, available on microfilm to order
() = volume (or part volume) sold separately
[] = only sold as part of a long set of volumes

REPRINTS AND MISCELLANEOUS PUBLICATIONS

PLANT PATHOLOGY COMMITTEE: *List of Common British Plant Diseases*, 3rd Ed. 1945. Price net, 5s. Postage, 4d.

A. A. PEARSON: *Cooke's illustrations of British Fungi* (Annotations by L. Quélet, R. Maire & C. Rea). Ex Vol. 20, Pt. 1. 1935. Price net, 2s. 6d. Postage, 3d.

T. PETCH: *British Hypocreales*. Ex Vol. 21, Pts. 3 & 4. 1938. Price net, 2s. Postage, 3d.

A. A. PEARSON & R. W. G. DENNIS: *Revised list of British Agarics and Boleti*. Ex Vol. 31, Pts. 3 & 4. 1948. Price net, 5s. Postage, 3d.

J. RAMSBOTTOM & F. L. BALFOUR-BROWNE: *List of Discomycetes recorded from the British Isles*. Ex Vol. 34, Pt. 1. 1951. Price net, 5s. Postage, 3d.

FIFTY YEAR INDEX TO THE TRANSACTIONS, Vols. 1-30; 1896-1946. Compiled by G. R. BISBY. Price net, 21s. Postage, 6d.

E. M. BLACKWELL: *Haustoria of Phytophthora infestans and some other species*. Ex Vol. 36, Pt. 2. 1953. Price 4s. Postage, 3d.

K. SAMPSON & J. H. WESTERN: *Diseases of British Grasses and Herbage Legumes*. 2nd Edition. 1954. Price, 15s. Postage, 6d.

Enquiries and orders for the above to be addressed to

CAMBRIDGE UNIVERSITY PRESS

200 EUSTON ROAD, LONDON, N.W. 1

or to

CAMBRIDGE UNIVERSITY PRESS

AMERICAN BRANCH, 32 EAST 57TH STREET, NEW YORK 22

FORAY COMMITTEE PUBLICATIONS

(To be obtained from the Treasurer and on sale at meetings and forays)

A key to the genera of the agarics and Boleti. By A. A. PEARSON. 32 pp. 1950. Price net, 2s. 6d. Postage, 2d.

Guide to the literature for the identification of British fungi. 1952. Price net, 2s. 6d. Postage, 2d.

Census Catalogue of British Lichens. By W. WATSON. 1953. Price 7s. 6d.

CONTENTS

	PAGE
<i>Gliocladium roseum</i> Bain., and its synonyms. By Ivor Isaac. (With 10 Text-figures)	193
<i>Cercospora elaeidis</i> leaf spot of the oil palm. By W. G. Kovachich. (With Plate 6 and 2 Text-figures)	209
The development of sclerotia of certain fungi. By Brenda B. Townsend and H. J. Willetts. (With 3 Text-figures).	213
Morphology and development of fungal rhizomorphs. By Brenda B. Townsend. (With 4 Text-figures)	222
<i>Candelabrum spinulosum</i> : a new British record. By T. L. C. Bottomley. (With 1 Text-figure)	234
Studies in the Myriangiales. IV. <i>Sphaceloma hesperethusae</i> sp.nov. on <i>Hesperethusa</i> and <i>Pleiospermium</i> . By Anna E. Jenkins and A. A. Bitancourt. (With Plates 7 and 8)	236
Aquatic predacious fungi. III. By Mary Peach. (With 6 Text-figures)	240
Notes on British Uredinales. By Malcolm Wilson and D. M. Henderson	248
Further descriptions of luminous agarics. By E. J. H. Corner. (With Plate 9 and 12 Text-figures)	256
<i>Ceriosporopsis halima</i> Linder and <i>Ceriosporopsis cambrensis</i> sp.nov.: two marine pyrenomycetes on wood. By Irene M. Wilson. (With 23 Text-figures)	272
Studies on a canker disease of cypresses in East Africa, caused by <i>Monochaetia unicornis</i> (Cooke & Ellis) Sacc. II. Variation in the morphology and physiology of the pathogen. By D. Rudd Jones. (With Plates 10 and 11, and 5 Text-figures)	286
Myxomycetes from Jamaica. By Const. J. Alexopoulos and E. S. Beneke.	306
Deme structure in higher fungi: <i>Mycena galopus</i> . By A. F. Parker-Rhodes.	314

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, Cambridge University Press, 200 Euston Road, London, N.W. 1, price 2s. 6d.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

The British Mycological Society subscribes to the Fair Copying Declaration, details of which may be obtained from the offices of the Royal Society.